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PLK1 facilitates chromosome biorientation by suppressing centromere disintegration driven by BLM-mediated unwinding and spindle pulling

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Centromeres provide a pivotal function for faithful chromosome segregation. They serve as a foundation for the assembly of the kinetochore complex and spindle connection, which is essential for chromosome biorientation. Cells lacking Polo-like kinase 1 (PLK1) activity suffer severe chromosome alignment defects, which is believed primarily due to unstable kinetochore-microtubule attachment. Here, we reveal a previously undescribed mechanism named ‘centromere disintegration’ that drives chromosome misalignment in PLK1-inactivated cells. We find that PLK1 inhibition does not necessarily compromise metaphase establishment, but instead its maintenance. We demonstrate that this is caused by unlawful unwinding of DNA by BLM helicase at a specific centromere domain underneath kinetochores. Under bipolar spindle pulling, the distorted centromeres are promptly decompacted into DNA threadlike molecules, leading to centromere rupture and whole-chromosome arm splitting. Consequently, chromosome alignment collapses. Our study unveils an unexpected role of PLK1 as a chromosome guardian to maintain centromere integrity for chromosome biorientation.
**Introduction**

Chromosome mis-segregation has wide implications in cancer and rare congenital disorders. To achieve faithful chromosome segregation, condensed chromosomes need to be properly aligned prior to disjunction through a mitotic process called chromosome biorientation. This requires a stable connection of spindle microtubules (MTs) emanating from opposite centrosomes to centromeres via the macromolecular complex of kinetochores (KTs). A single unattached chromosome can activate the spindle assembly checkpoint (SAC), inhibiting the anaphase promoting complex/cyclosome (APC/C) and hence blocks anaphase onset. This elegant system allows cells to correct possible KT-MT attachment errors and prevent chromosome mis-segregation. During chromosome biorientation, centromeres and kinetochores are inevitably under constant spindle pulling tension, due to the persistence of sister chromatid cohesion. The centromere architecture is presumably maintained through chromosome condensation, whilst the KT-MT stable attachment requires activity of a key mitotic kinase, Polo-like kinase 1 (PLK1). In early mitosis, PLK1 localises predominantly at kinetochores and centrosomes. Inactivation of PLK1 has been shown to induce severe chromosome misalignment, which is generally attributed to a failure in building stable KT-MT attachment. However, how PLK1 promotes chromosome biorientation still requires investigation.

Once biorientation is achieved on every chromosome, the spindle checkpoint is satisfied. This leads to the activation of APC/C and cleavage of cohesin, allowing the poleward movement of sister chromatids. Interestingly, studies show that despite their separation, sister chromatids can remain intertwined by DNA linkage molecules that manifest as so-called ultrafine DNA bridges (UFBs). Generally, UFBs are thought to be unresolved double-stranded DNA catenanes, especially those that arise at centromeres. However, studies have also shown that incomplete replication intermediates and homologous recombination (HR) structures can give rise to UFB structures. Regardless of their origins, ultrafine DNA bridges are recognised by a UFB-binding complex comprising of PICH (Plk1-interacting checkpoint helicase) translocase, BLM (Bloom's syndrome) helicase and its interacting factors, including TOP3A and TOP2. However, the precise molecular mechanism of UFB resolution is not yet fully understood.

Chromosome biorientation not only plays a critical role to ensure equal chromosome segregation, but also facilitates the regulation of the spindle checkpoint and mitotic progression. Many studies have shown that PLK1 is essential for chromosome
biorientation; however, the underlying mechanism(s) is still not fully clear. In the current study, unexpectedly, we find that PLK1 in fact can protect centromere integrity for chromosome biorientation maintenance. We demonstrate that in the absence of PLK1, the UFB-binding complex aberrantly targets and unwinds centromeres, leading to their rupture in concerted action with bipolar spindle pulling. As a consequence, cells lose centromere integrity and fail to maintain metaphase alignment. Therefore, our study provides an alternative mechanism of chromosome misalignment in PLK1-defective cells. Importantly, it also reveals a previously undescribed pathway of centromere protection during mitosis.

Results

PLK1 inactivation leads to collapse of metaphase alignment

It is well documented that cells cannot achieve proper chromosome alignment without PLK1 activity6. Consistent with this, inhibition of PLK1 using a well-characterised small molecule inhibitor, BI2536 (IC50 = 0.83nM)20, induced severe chromosome misalignment in hTERT-immortalised human RPE1 cells. The BI2536-induced mitotic arrest manifested in a way similar to treatments of the spindle poison (nocodazole) and kinesin inhibitor (Monastrol) (Supplementary Figure. 1a). However, live-cell time-lapse microscopy on pre-synchronised RPE1 cells revealed that, unlike nocodazole and monastrol treatments, BI2536 did not fully prevent chromosome congression (Fig. 1a and Supplementary Figure. 1b). Nearly 80% of BI2536-treated RPE1 cells managed to align their chromosomes in the metaphase plane, but shortly after, succumbed to a loss of maintenance; namely chromosomes drifting away from the equator and scattering into a ‘Fig-8’ or ‘polo’21–like pattern (Figs. 1a-c, Supplementary Figure. 1c and Supplementary Movie 1). We referred to this phenomenon as ‘metaphase collapse’. In contrast, cells treated with the APC/C inhibitor, ProTAME, remained arrested at metaphase for extended periods (Fig. 1c).

Formation of centromeric DNA linkages between chromosomes

Strikingly, in the metaphase-collapse cell population, we observed a threadlike structure that was decorated by the PLK1 protein (Fig. 2a; arrows). It was not present in DMSO-treated pre-anaphase cells (i.e. prometaphase and metaphase) (Fig. 2a). As the threadlike structure was reminiscent of anaphase ultrafine DNA bridges (UFBs)10,11, we investigated whether they were DNA molecules; or a mis-localisation of PLK1 to cytoskeleton structures. Immunofluorescence co-staining showed that PICH translocase, a well-known UFB marker, was present along the PLK1-coated threads induced by BI2536 (Fig. 2b and
Supplementary Figure. 1d). Furthermore, other known UFB-associated factors including BLM helicase and replication protein A (RPA) were also present (Fig. 2c). It is worth noting that RPA decorates the threads without necessarily following the PICH/BLM signals, and it can also be found on regions where no or weak PICH/BLM signals were detected (Fig. 2c; arrows). Similar results were obtained by using different antibodies against BLM and different subunits of RPA (Fig. 2d). This localisation pattern is similar to recent reports showing the binding of RPA to stretched DNA molecules, or DNA bridges, is not always coupled with the PICH/BLM complex\(^{12, 22}\). Therefore, the RPA association likely represents the presence of single-stranded DNA. To validate the immunofluorescence staining results, we examined Bloom's syndrome fibroblast cells stably expressing a GFP-tagged BLM, and RPE1 cells expressing a GFP-tagged PLK1. We found both GFP-tagged proteins were also present along the thread molecules induced by BI2536 (Supplementary Figures. 1e, f; arrows). In addition to this, we also found that PLK1 indeed associated with UFBs in anaphase cells (Supplementary Figure. 1g; arrows). Together, these data suggest that the BI2536-induced thread molecules are highly likely a form of DNA structure; and possibly composed of both single-stranded and double-stranded DNA. As predicted, the threadlike structures did not co-localise with mitotic microtubules (Supplementary Figure. 1h).

Next, we investigated the origin of the DNA threads. We found that all of the DNA threads analysed linked to centromeric regions, either through one or both of their termini (Fig. 2e). In some optical sections, it was apparent that two separating centromeres were interconnected by a DNA thread (Fig. 2e; arrows). Since PLK1 inhibition prevents anaphase onset, these DNA threads cannot be explained as the centromeric UFBs coming from disjoined sister chromatids. However, another possibility is that PLK1 inhibition might induce precocious sister chromatids separation; an effect similar to Shugosin 1 (SGO1) depletion\(^{23, 24}\) (Supplementary Figure. 2a), which exposes UFBs before anaphase\(^{11}\) (Supplementary Figure. 2b; arrows). This proposal, however, is very unlikely because PLK1 has been shown to be required for the release of arm cohesin; its inactivation in fact blocks premature loss of cohesion\(^{25-27}\). Consistent with these studies, we confirmed that BI2536 did not induce premature separation of sister chromatids in RPE1 cells (Supplementary Figure. 2c). More importantly, by co-staining with topoisomerase 2alpha (TOP2A), a chromatid-axis marker, we visualised that cohesed chromosomes were linked by the DNA threads, induced by PLK1 inhibition (Fig. 2f; arrows). Therefore, the absence of PLK1 activity leads to the formation of a DNA linkage structure that strikingly connects centromeres between cohesed chromosomes. Because of the concomitant occurrence of
metaphase collapse and centromere DNA linkages, we speculate that the failure of chromosome alignment in PLK1-inactivated cells may not be merely attributed to unstable KT-MT attachments as previously thought.

**Mitotic loss of PLK1 causes centromeric DNA linkages**

The DNA linkages induced by BI2536 arise predominantly at centromeres – a genomic region composed of highly repetitive sequences. We sought to test if they might be caused by potential disturbance of DNA replication (or homologous recombination) during the course of BI2536 treatment. We used EdU labelling to distinguish between cells that were in an ongoing, or post DNA replication stage, whilst under BI2536 treatment (Supplementary Figure. 3a). If centromere DNA threads are a by-product of abnormal DNA replication, we expect to observe their formation only in the EdU-positive, but not negative, mitotic population. Contrary to this hypothesis, we found that the majority of EdU-negative mitotic cells (69±4%), which were presumably in G2/M while BI2536 was applied, remained positive for DNA thread formation (Supplementary Figure. 3b). Moreover, inhibition of PLK1 in early mitotic RPE1 cells obtained through a release from RO3306-induced G2 arrest also induced centromere DNA threads (Supplementary Figure. 3c). In addition, treating asynchronous RPE1 cells with BI2536 for 1 hour also caused centromere DNA thread formation, albeit with a lower frequency (Supplementary Figure. 3d). These data indicate that the formation of centromere DNA linkages likely results from a loss of M-phase specific function of PLK1. However, thymidine pre-treatment and/or synchronistic mitotic entry may enhance the phenotype appearance.

Using the same treatment protocol, we also found that BI2536-induced centromere DNA threads in all other examined cell types, but with different frequencies (Supplementary Figure. 4a). They included 1BR3 primary fibroblasts (31%), 82-6 hTERT-immortal fibroblasts (24%), HCT116 colon (69%) and HeLa cervical cancer cells (21%). Since the DNA thread formation occurs following metaphase collapse, the different frequencies between cell lines, (e.g. RPE1 vs HeLa), may relate to their ability to establish metaphase. In agreement with this, time-lapse microscopy revealed that, as compared to RPE1, HeLa cells poorly progressed into a metaphase(-like) stage under BI2536 treatment (Supplementary Figure. 4b). This data is consistent with other studies\(^{27, 28}\), and may indicate that the formation of bipolar spindle attachment in HeLa cells is more sensitive to the loss of PLK1 activity.

**PLK1 inactivation induces whole-chromosome arm splitting**
As shown above, centromere DNA threads cannot be described as originating from the DNA entanglements between sister chromatids/centromeres. We thus investigated other possible cause(s). PICH translocase binds with a high affinity to DNA molecules under tension\textsuperscript{29}. This could indicate that the DNA threads may be a form of abnormally stretched centromeric chromatin. Interestingly, we detected activation of DNA damage responses at (peri)centromere regions, as labelled by $\gamma$H2AX staining, following BI2536 treatment (Supplementary Figure. 5a). The damage response was mainly observed in metaphase collapse populations rather than in early mitotic cells (e.g. prophase/early prometaphase) (Supplementary Figure. 5a). As expected, $\gamma$H2AX was mostly not detected at (peri)centromere regions in DMSO-treated mitotic cells (Supplementary Figure. 5b).

To determine if this was caused by chromatin damage, we examined mitotic chromosome spreads (Supplementary Figure. 6a). In control RPE1 cells (DMSO- and nocodazole-treated), their chromosomes displayed normal configurations and their average numbers were very close to 46 (diploid) (Supplementary Figures. 6b, c). In contrast, chromosomes of BI2536-treated cells exhibited a shorter and more compact structure, but strikingly, their chromosome numbers increased to an average of 59 (Supplementary Figures. 6b, c). This increment cannot be explained by chromosome mis-segregation, because PLK1 inactivation blocks anaphase onset. Thus, a plausible explanation is chromosome fragmentation. Moreover, we found that the increase in chromosome numbers in BI2536-treated cells was suppressed by co-treatment with nocodazole (Supplementary Figures. 6b, c), implying a spindle (or tension)-dependent process. Furthermore, centromere-telomere fluorescence in situ hybridization (ctFISH) analysis confirmed that the mitotic chromosomes in BI2536-treated were indeed broken (Fig. 3a and Supplementary Figure. 7). Notably, the broken chromatin largely resembled telocentric chromosomes; namely the centromere residing at one end of the chromatin, but lacking the telomere signals (Fig. 3a, middle panels – asterisks and Supplementary Figure. 7a, middle panels). This pattern suggests that the breakage occurs either at, or very close to the centromere. Supporting this, we also observed partial centromere splitting (Fig. 3a, middle panels – arrowheads and Supplementary Figure. 7a), and occasionally, saw a CEN DNA thread linking two separating broken chromosome arms (Fig. 3a, middle panels – connecting arrow and Supplementary Figure. 7a). Nocodazole treatment again suppressed chromosome arm breakages, but seemed to have a lesser effect on the partial splitting of centromeres (Fig. 3b). Together, these results demonstrate that the loss of PLK1 activity induces centromere rupture in a spindle-dependent manner. In agreement to this, we found that nearly all the broken chromatin (99.6%) retained centromere sequences (Fig. 3c and
Supplementary Figure. 7b), indicating that most, if not all of the breakages, occur within the core centromere.

Our results from both cytological and cytogenetics analyses suggest that the centromere DNA threads induced by PLK1 inhibition are highly likely caused by abnormal stretching of the core centromere chromatin by the spindle pulling forces. As predicted, nocodazole suppressed both centromere splitting and DNA thread formation (Fig. 3b and Supplementary Figure. 8). Given that centromeric DNA threads arise mostly after metaphase establishment, we postulated that rather than by spindle-dependent chromosome movement, they are likely mediated by the tension exerting across the centromeres due to ‘bipolar’ spindle attachment. We thus used Monastrol, the Eg5 inhibitor, to prevent bipolar spindle establishment while keeping microtubule attachment30. As predicted, ‘monopolar’ spindle attachment is not sufficient to induce centromere DNA threads (Supplementary Figure. 8). Therefore, centromere splitting requires bipolar spindle pulling forces.

To our knowledge, this striking phenomenon of spindle-mediated centromere rupture has never been described; we thus termed this ‘centromere dislocation’. Using multi-colour FISH (mFISH) analysis, we further validated that PLK1 inactivation can cause whole-chromosome arm separation (Fig. 3d). In some cases, the separated whole-arms were located in close vicinity (Fig. 3d; e.g. chromosomes 7p-7q, 12p-12q, 17p-17q), which may imply a residual physical connection, presumably through the ultrafine centromeric DNA threads. In addition, centromere dislocation tended to occur more frequently on longer chromosomes (Fig. 3d, inset). Collectively, our data shows that, in the absence of PLK1 activity, centromere chromatin fails to withstand bipolar spindle tensions and the core axis is transformed into an ultrafine DNA threadlike structure. Indeed, we were able to detect condensin, a chromosome axial element, associating along the stretched DNA threads (Fig. 3e; arrows). The disintegration of centromeres therefore causes whole-chromosome arm splitting and explains why cells simultaneously lose their metaphase alignment (Fig. 3f).

**PLK1 kinase activity suppresses centromere disintegration**

Thus far, most of the experiments were carried out using the PLK1 inhibitor, BI2536. To rule out potential off-target effects, such as inhibition to other PLK members30, we employed an engineered RPE1 cell line in which the endogenous wildtype PLK1 has been replaced with an analogue-sensitive allele, PLK1as. The catalytic cavity of the PLK1as
protein has been modified such that it no longer binds to BI2536; instead only to the unrelated ATP-analogue, 3-MB-PP1 (Supplementary Figure 9a). As predicted, BI2536 failed to induce metaphase collapse and centromere DNA thread formation in the engineered PLK1as cells. Importantly, these mitotic defects were recapitulated by using 3-MB-PP1 analogue (Supplementary Figures 9a-d). In addition, depletion of the PLK1 protein in RPE1 cells by RNA interference (RNAi) also induced centromere DNA threads and dislocations (Supplementary Figures 9e-f), which further rules out the potential dominant effect as a result of trapping an inactive form of PLK1 onto chromatin by the small molecules. Therefore, PLK1 kinase activity per se is essential to suppress centromere disintegration.

**Aberrant association of UFB-binding factors to kinetochores**

The failure of centromeres to withstand bipolar spindle pulling in the absence of PLK1 function might indicate that centromere chromatin structure is impaired. We thus analysed the centromeres in the BI2536-treated RPE1 cells before metaphase collapse occurs. We found that there was a progressive formation of RPA foci at or near kinetochores; from early prometaphase to metaphase(-like) stages (Fig. 4a). This was also sensitive to nocodazole treatment (Fig. 4a). In control, we rarely detected RPA foci at centromeres in normal metaphase cells (Fig. 4b). More interestingly, we also found increased accumulations of BLM and PICH foci at or near the kinetochores in the metaphase(-like) cells, again only after BI2536 treatments (Fig. 4c and Supplementary Figure 10a). Occasionally, PICH was found at the inner centromeres of untreated cells (Supplementary Figure 10a; yellow arrows), perhaps reflecting the unresolved DNA entanglements between sister centromeres as proposed previously. Earlier studies have reported that (phospho)-RPA and BLM foci are observed at centromeres of cytospun chromosomes. However, under our experimental conditions, both RPA and BLM foci were rarely detected at centromeres in normal intact mitotic cells (Figs. 4b, c). In contrast, PICH foci were consistently visualised at kinetochores in normal mitotic cells (Supplementary Figure 10a). To confirm that PLK1 inactivation also enhances PICH loading, we performed quantitative imaging analysis on co-cultured RPE1 cells, using a mixture of cells expressing either a wildtype PLK1 or GFP-tagged PLK1as protein. Under BI2536 treatment, there was a marked increase in both intensity and number of PICH foci at kinetochores in wildtype PLK1, but not in the GFP-PLK1as cells (Supplementary Figures 10b-d). Conversely, 3-MB-PP1 induced PICH accumulation at kinetochores in the GFP-PLK1as cells (Supplementary Figures 10b-d). As expected, PICH, BLM and RPA foci
were mostly co-localised at kinetochores (Supplementary Figure 10e; arrows). Therefore, the UFB-binding complex is aberrantly recruited to kinetochore regions when PLK1 function is compromised. Since BLM and PICH possess activities of DNA unwinding and of DNA displacement, respectively, this led us to speculate that the increase in centromeric RPA foci formation may be due to illegitimate DNA unwinding.

**Centromere distortion underneath kinetochores**

The localization of PICH to kinetochores is independent of PLK1 (Supplementary Figure 9e). Our data show that inactivating PLK1 even increases the binding of PICH, BLM and RPA to kinetochores. Whether the complex actually targets the centromere chromatin, or is aberrantly enriched at kinetochores is unclear. To address this, we employed high-resolution microscopy to precisely locate the complex within the territory of centromeres (Supplementary Figures 11a). We found that PICH localised in centromeres at a position ~160nm away from the outer kinetochore component, as marked by NUF2 (Supplementary Figures 11b, d). In a control measurement, the inner KT component, CENPA, was mapped ~100nm inwards from NUF2 in metaphase cells (Supplementary Figures 11c, d). The CENPA-NUF2 distance was reduced (~80nm) in anaphase cells (Supplementary Figures 11c, d), probably due to a reduction of intra-kinetochore tension following sister chromatids cohesion loss. This inward position of PICH suggests that it likely locates at centromere chromatin. Further co-staining of PICH and CENPA confirmed that PICH resides at a centromeric domain ~100nm beneath CENPA (Figs. 4d and Supplementary Figure 11d). Likewise, both BLM and RPA foci, were mapped underneath CENPA, with distances of ~120 and ~150nm, respectively (Figs. 4d and Supplementary Figure 11d). All of these proteins displayed mirror localization patterns, reflecting a typical symmetry of sister centromere-kinetochore organisation. We referred to this specific centromere site as ‘k-kinetochore-chromatin’ or ‘K-chromatin’ (Fig. 4e).

The K-chromatin localisation finding is consistent with our notion that centromeric chromatin is probably targeted by the UFB-binding complex after PLK1 inhibition. If the increased formation of RPA foci at K-chromatin reflects aberrant DNA unwinding, this may weaken centromere rigidity to counteract spindle pulling forces. Notably, we observed detachments of kinetochore complex in a small population of centromeres (<4%) in metaphase(-like) cells prior to collapse. Intriguingly, some of the kinetochores remained connected by a short thread, as labelled by PICH or RPA staining (Figs. 5a, b; arrows). Centromere staining was sometimes evident along the short thread (Fig. 5a;
arrows), implying a protrusion of centromeric DNA. We postulated that this might be the early sign of centromere disintegration. Further analysis of the centromere-kinetochore integrity revealed that there was a large percentage of centromeres losing one of the two sister kinetochores after metaphase collapse (Fig. 5b-d). The side of the centromere where a kinetochore is missing was concomitant with the formation of DNA thread linkages (Fig. 5c; connecting arrows). Therefore, there are apparent alterations on the centromere-kinetochore configuration prior to and during centromere disintegration.

BLM helicase activity mediates centromere disintegration

Next, we examined whether centromere disintegration is mediated by BLM and PICH. We knocked down BLM by RNAi before BI2536 treatment (Fig. 6a). Silencing BLM for longer than 48 hours in RPE1 cells reduced the efficiency of thymidine release, therefore we treated cells with siBLM oligos for only 24 hours prior to G1/S release (Supplementary Figure. 12a). Despite partial depletion (Supplementary Figures. 12b-d), we found that first, BLM knockdown significantly reduced the formation of RPA foci at K-chromatin induced by BI2536 (Fig. 6b); second, it also diminished both centromere DNA thread formation and centromere dislocation (Supplementary Figs. 12e, f). BLM depletion did not impair PICH centromeric localisation (Supplementary Figure. 12g), suggesting that, without BLM, PICH alone is not sufficient to drive centromere disintegration. To confirm the specificity of BLM knockdown, we performed our analyses on HAP1 cells in which the endogenous BLM was knocked out by CRISPR genome editing (Supplementary Figures. 13a, b). Consistently, BLM knockout abolished centromere DNA thread formation and centromere dislocations (Figs. 6c, d). Though, occasionally, chromatid breaks were observed in ΔBLM HAP1 cells, the breakpoint was not at the centromere (Fig. 6d, arrow). Therefore, in addition to the bipolar spindle pulling forces, BLM is a key driver of centromere disintegration after the loss of PLK1 activity.

Centromere disintegration might be initiated through unlawful DNA unwinding by BLM. Thus, we determined if BLM’s helicase activity is required. We generated polyclonal cell lines from the ΔBLM HAP1 cells, which stably express either a GFP-tagged wildtype (WT) or a helicase-dead (Q672R) BLM protein. The expression of the GFP-Q672R protein was similar to the endogenous BLM level in HAP1 cells; whereas, the GFP-WT was over-expressed (Supplementary Figure. 13b). In agreement with our notion, the helicase-dead (Q672R) BLM failed to induce centromere DNA threads and dislocations caused by BI2536 treatments (Figs. 6c-d). However, as the expression level of the GFP-Q672R mutant was lower than the wildtype control; to perform a better comparison, we re-
sorted the wildtype GFP-BLM cells to obtain a cell population with a lower BLM expression (Supplementary Figure. 13c). Despite a much lower abundance, the wildtype GFP-BLM protein was still capable of driving centromere DNA thread formation (Supplementary Figure. 13d). More importantly, the GFP-Q672R mutant protein no longer induced RPA foci formation, despite its aberrant enrichment at centromeres following PLK1 inhibition (Fig. 6e). Therefore, we conclude that centromere disintegration is mediated by BLM-mediated DNA unwinding at centromeres.

Next, we investigated the role of PICH. Knockdown of PICH, like BLM, also suppressed BI2536-induced centromere DNA thread formation and centromere dislocations (Figs. 7a-c). However, it also abolished BLM localisation and RPA formation at K-chromatin (Fig. 7d). Therefore, PICH acts upstream to facilitate the recruitment of BLM to centromeres after PLK1 inactivation. Taken together, our data suggest that PLK1 has an important function to protect centromeres from unlawful DNA unwinding, mediated by the PICH/BLM complex. The structural change probably impairs centromere rigidity and causes the failure to withstand bipolar spindle pulling forces. Consequently, centromeres are torn apart, leading to whole-chromosome arm splitting and chromosome biorientation failure.

**Centromeric tethering of BLM does not induce metaphase collapse**

Both PICH and BLM interact with PLK1 and are hyperphosphorylated during mitosis\(^1\)\(^{10}\),\(^{39}\),\(^{41}\). Hyperphosphorylation of PICH and BLM is partially dependent on PLK1\(^1\)\(^{42}\) (Supplementary Figure. 14). It has been proposed that hyperphosphorylation of BLM can prevent its association with mitotic chromosomes\(^4\),\(^1\),\(^{43}\). Thus, we sought to test whether the abnormal loading of BLM to centromeres, presumably due to the loss of PLK1-mediated phosphorylation, might cause centromeric DNA unwinding and dislocation. We tethered BLM to centromeres in HeLa cells by fusing a truncated CENPB (1-158) to a GFP-tagged BLM. Transient expression of the wildtype GFP-BLM and the CENPB-GFP-BLM fusion proteins showed that the wildtype GFP-BLM exhibited diffused localisation pattern and was mostly excluded from mitotic chromosomes after nuclear envelope breakdown. In contrast, the CENPB-GFP-BLM fusion protein was enriched at core centromeres throughout mitosis (Supplementary Figure. 15a). However, we did not find that tethering BLM to centromeres induced obvious mitotic defects such as mitotic arrest, as observed by PLK1 inhibition. Time-lapse live-cell imaging showed that the CENPB-GFP-BLM transfected cells, like the wildtype GFP-BLM, progressed successfully into anaphase, without metaphase collapse (Supplementary Figure. 15b). Moreover, RPA (ssDNA)
formation was not detected at centromeres where the CENPB-GFP-BLM protein was enriched (Supplementary Figure. 15c). Therefore, artificially over-loading BLM at centromeres seems not sufficient to trigger DNA unwinding and centromere disintegration when PLK1 remains active. Though speculative, the triggering of centromere dislocation in PLK1-inactivated cells might be caused by mis-regulation of BLM (and PICH) activity; and/or because of improper formation of centromere structures that mis-activates the PICH/BLM complex prior to chromosome disjunction.

**Constitutive PLK1 activity for centromere integrity maintenance**

To test if centromere disintegration might be caused by centromere malformation during early mitosis, we inhibited PLK1 only after mitotic cells had fully formed their chromosomes and progressed into metaphase, whilst in the presence of active PLK1. RPE1 cells stably expressing a GFP-tagged PLK1 were first blocked at metaphase using the APC/C inhibitor, ProTAME. Time-lapse live-cell imaging recorded that upon the addition of BI2536, the fully bi-oriented chromosomes started losing their alignment. Most importantly, this was accompanied by the formation of DNA threads (Fig. 8a and Supplementary Movies 2 &3), indicating the occurrence of centromere dislocation. Furthermore, we found that centromere dislocation can happen rapidly, as within 30 minutes of BI2536 addition, more than 60% of the metaphase-arrested cells generated centromere DNA threads (Fig. 8b). As centromere dislocation depends on bipolar spindle pulling, this would imply that the kinetochore-microtubule attachment is not instantly destroyed, at least in those centromeres with DNA threads. Therefore, rather than due to an initiate malformation, centromere disintegration is likely triggered because of a defect in centromere structure maintenance.

**Depletion of PICH and BLM prolongs metaphase alignment**

Thus far, our data indicates that apart from the proposed model of KT-MT destabilisation, a failure in centromere integrity maintenance is another cause of chromosome misalignment. To further test this, we examined if suppression of centromere dislocation, by PICH and BLM depletion, might rescue the metaphase alignment defect in PLK1-inhibited cells. Knocking down PICH or BLM had no adverse effect on metaphase establishment in RPE1 cells under BI2536 treatments. However, it significantly prolonged the metaphase(-like) stage as compared to control cells (Fig. 8c and Supplementary Movies 4-6). Although the metaphase chromosomes inevitably misaligned after long delays in the PICH/BLM-depleted cells, they dispersed more like a ‘polo’ pattern, rather than the ‘Fig-8’ collapsed shape. As we showed that PICH/BLM depletion abolished
centromere dislocations, we believe that the ultimate alignment failure is likely caused by KT-MT destabilisation. Nevertheless, it seems that even in the absence of PLK1 activity, the centromeres and kinetochores remain competent to support chromosome biorientation, at least in RPE1 cells, as long as the PICH/BLM complex is inactivated. Moreover, our data also implies that KT-MT destabilisation, if it occurs, seems at a relatively slow rate as compared to centromere disintegration.

In summary, we report an unexpected role of PLK1 during chromosome biorientation, which prevents centromeres from destruction, mediated by the co-action of DNA unwinding by BLM helicase and bipolar spindle pulling (Fig. 9).

Discussion

One of the key mitotic functions of PLK1 is to promote stable attachments between spindle microtubules and kinetochores6. In the current study, we reveal a hitherto undescribed role of PLK1 as a centromere guardian for chromosome alignment. We show that the lack of PLK1 activity leads to the failure of centromeres to withstand bipolar spindle pulling tension. As a consequence, centromere chromatin is stretched into a threadlike structure, resulting in centromere splitting, whole-chromosome arm separation and loss of metaphase alignment. Further experiments demonstrate that the disintegration of centromeres is not a passive process, but is actively driven through illegitimately unwinding of centromeric DNA by the PICH/BLM complex. Our results highlight a PLK1-dependent pathway for centromere maintenance during mitosis.

BLM is the key molecular driver of centromere disintegration, but it remains unclear how PLK1 counteracts its mediated destruction. Given that both BLM and PICH proteins are substrates of PLK1, a reasonable speculation is that PLK1 can regulate the activity of PICH/BLM complex during mitosis. Previous studies have shown that before anaphase onset, BLM poorly associates with mitotic chromosomes41,43 and on ultrafine DNA bridges generated from prematurely disjoined sister chromatids11. The chromatin exclusion of BLM, presumably by hyperphosphorylation, could limit its DNA transaction activity. However, artificially tethering BLM to centromeres is not sufficient to induce DNA unwinding and centromere dislocation, which may suggest that either the BLM protein remains inactive, or additional factors such as PICH activation and/or chromatin remodeling are required. Alternatively, PLK1 may protect centromeres through facilitating normal condensation of centromeres, a process if compromised might create a DNA substrate that mis-activates the PICH/BLM complex. However, impairing
chromosome condensation by condensin depletion, which leads to abnormal stretching of sister centromeres\textsuperscript{44,45}, does not trigger similar phenotypes of centromere rupture and chromosome misalignment as induced by PLK1 inhibition. Additionally, the fact that centromere disintegration can be induced in ‘mature’ mitotic cells; namely those cells that have fully formed normal metaphase, would suggest that rather than due to an initial chromatin malformation, it is probably caused by centromere maintenance impairment. Nevertheless, no matter if there is a structural defect, loss of PLK1 does not seem to greatly compromise chromosome biorientation, at least in RPE1 cells, as long as the PICH/BLM complex is inactivated. We postulate that PLK1 may protect centromeres through both chromatin structure maintenance and the regulation of the PICH/BLM complex activity. Further experiments will need to dissect the underlying mechanism(s).

Another very intriguing finding is that when acting in concert with bipolar spindle tension, the PICH/BLM complex can promote decompaction of the centromere axis. This converts the centromeric chromatin into an ultrafine DNA structure, reminiscent of anaphase UFBs\textsuperscript{10,11}, leading to whole-chromosome arm separation. Conceivably, if such decompaction activity is applied at a chromosomal region where sister DNA intertwinements persist, it may be able to relieve the entangling constraints and facilitate the poleward separation of sister chromatids during anaphase. Though this is speculative, this finding could provide an alternative clue to understand how the UFB-binding complex may function during chromosome disjunction, and potentially also explain why a long region of UFBs is always coated by the PICH/BLM complex. In principle, such powerful action would need to be under a tight control before anaphase onset, otherwise it could lead to pathological damage at chromatin sites where tension is exerted; namely the centromere. Finally, the identification of a centromere-specific breakage pathway, independent of chromosome mis-segregation\textsuperscript{46,47}, also offers an alternative direction in understanding the origin of complex chromosome rearrangements, such as whole-chromosome arm rearrangements, which are observed in many human tumours and rare genetic disorders\textsuperscript{48-51}.

In conclusion, our study unveils an unexpected participation of PLK1 and the UFB-binding complex in the safeguard of centromere integrity during mitosis, which is critical for faithful chromosome segregation and chromosome stability.
Methods

Cell culture
RPE1-hTERT, 82-6-hTERT normal diploid cell lines, 1BR3 primary fibroblasts, HCT116 colon and HeLa cancer cells were obtained from the Genome Damage and Stability Centre (GDSC) Cell Bank. All cell lines were authenticated by STR genotyping from European Collection of Cell Cultures. RPE1-hTERT derivative cells were generated and supplied by Mark Burkard (University of Wisconsin). Bloom’s syndrome fibroblasts (GM08505) were obtained from Phillip North (University of Oxford). HAP1 cells and HAP1ΔBLM cells were obtained from Marcel van Vugt (University of Groningen). All cell lines passed mycoplasma tests (Lonza MycoAlert kit). RPE1-hTERT and its derivative cells were grown in DMEM/F-12 medium (Sigma) containing 15% foetal calf serum (FCS) and Pen/Strep antibiotics (P/S). 82-6 fibroblast cells were grown in DMEM/F-12 medium containing 15% FCS and P/S. HAP1 cells were grown in IMDM (Gibco) containing 10% FCS and P/S. 1BR3 primary cells were grown in MEM (Gibco) containing 2mM L-glutamine, 15% FCS and P/S. HCT116 cells were grown in McCoy’s 5A (Gibco) containing 15% FCS and P/S. Bloom’s syndrome fibroblasts (GM08505) were transfected with a pEGFP-hBLM construct and selected by 700 µg/ml G418 for 14 days. A single clone was isolated and maintained in MEM (Gibco) containing 2mM L-glutamine, 10% FCS, P/S and G418. Cell cultures were maintained at 37°C in a humidified atmosphere containing 5% CO2. GFP-BLM(WT) and GFP-BLM(Q672R) HAP1 cells were generated by stable transfection with the corresponding constructs in HAP1ΔBLM cells by using FuGene HD (Promega) according to the manufacturer’s guidelines. The DNA constructs were created by sub-cloning EGFP-hBLM (WT) or Q672R (helicase dead mutant) fragments into a pSYC-181-(NEO) vector. Following a 1.2mg/ml of G418 selection for 14 days, GFP positive populations were sorted and isolated using a FACS cell sorter (BD FACS Melody).

Cell synchronisation and drug treatments for mitotic cell analysis
Cells were treated with 2mM of thymidine for 18 hours to enrich cells at the G1/S boundary. Cells were then released into S-phase by washing three times with pre-warmed culturing medium, or pre-warmed 1xPBS and released into fresh medium. Five to six hours post G1/S release, indicated inhibitors were added. At approximately 8-9 hours post the G1/S release, mitotic cells were fixed or enriched for analyses.

RNA interference
Cells were transfected with siRNA oligonucleotides using Lipofectamine RNAiMAX transfection reagent (Thermo Fisher Scientific) following the manufacturer’s guidelines. Cells underwent 1 or 2 rounds of siRNA transfection as necessary.
Non-targeting siRNA pool (Dharmacon ON-TARGET plus Non-targeting Pool – D-001810-10-05. UGGUUUACAUGUCGACUAA; UGGUUUACAUGUUGUGA; UGGUUUACAUGUUUUCUGA; UGGUUUACAUGUUUUCUGA)
PLK1 siRNA sequence (Dharmacon ON-TARGET plus SMARTpool – L-003290-00-0005. GCACAUACCACGCUUGACGUCU; CCACCAAGGUUUUCGAAUG; GCUCUUAUGACAUACCA; UCUCAGCGCCUCUAAUAG)
Sgo1 siRNA sequence (Dharmacon ON-TARGET plus SMARTpool – L-015475-00-0005.
CAGCCAGCGUGAACUAUAA; GUUACUAUCUCACAUGUCA; AAACGCAGGUCUUUUAUAG;
GUGAAGGAAUUCACCAGAA)
BLM siRNA sequence (Dharmacon ON-TARGET plus Individual – J-007287-08-0005.
GGAUGACUGAAUGGUUA)
PICH siRNA sequence (Invitrogen - AAUUCGGUAAACUCUAUCCACAGCU)

**Fluorescence immunostaining**
For immunostaining analyses, cells were seeded onto No. 1.5 or No. 1.5H cover glass and
fixed with Triton X-100-PFA buffer (250 mM HEPES, 1xPBS, pH7.4, 0.1% Triton X-100,
4% methanol-free paraformaldehyde) at 4°C for 20mins, or with PBS-PFA buffer (1xPBS,
4% methanol-free paraformaldehyde) at room temperature for 10mins. Pre-extraction
was carried out in indicated experiments before fixation by incubation of the cover glass
in pre-extraction buffer (20mM HEPES pH7.4, 0.5% Triton X-100, 50mM NaCl, 3mM
MgCl2, 300mM sucrose) for 10-15secs. Cells were incubated in permeabilisation buffer
(0.5% Triton X-100, 1XPBS) for 20mins on ice followed by blocking with foetal calf serum
for 15mins at room temperature. Cells were incubated with primary antibody at 37°C for
90mins followed by secondary antibody incubation at room temperature for 30mins.
Slides were washed with 1X PBS for 5 times at room temperature after antibody
incubation. Cells were mounted using DAPI-containing Vectashield mounting medium.

Primary antibodies used: anti-PICH (Abnova; H00054821-B01P, 1:100), anti-PICH
(Abnova; H00054821-D01P, 1:100), anti-BLM (Santa Cruz; sc-7790, 1:50), anti-BLM
(Abcam; ab21779, 1:200), anti-γH2AX (Upstate; JBW-301, 1:400), anti-TOP2A (Santa Cruz;
sc-5348, 1:100), anti-SMC2 (Bethyl Lab; A300-058A, 1:200), anti-RPA70 (Abcam;
ab79398, 1:200), anti-RPA32 (Abcam; ab2175, 1:200), anti-CENPA (Abcam; ab13939,
1:100), anti-CENPB (Abcam; ab25734, 1:800), anti-NUF2 (Abcam; ab122962, 1:200),
anti-PLK1 (Santa Cruz; sc-55504, 1:100), anti-pericentrin (Abcam; ab4448, 1:400), anti-
centromere (ImmunoVision; HCT-0100, 1:400) and GFP booster (ChromoTek; gba-488,
1:200). Secondary antibodies used: donkey anti-mouse Alexa Fluor 488, 555 and 647;
donkey anti-rabbit Alexa Fluor 488, 555 and 647; donkey anti-goat Alexa Fluor 488 and
555; goat anti-human DyLight 550 and 650 (All secondary antibodies are purchased from
ThermoFisher and used at 1:500 dilution).

**High-resolution deconvolution microscopy**
Images were acquired under a Zeiss AxioObserver Z1 epifluorescence microscopy system
with 40x/1.3 oil Plan-Apochromat, 63x/1.4 oil Plan-Apochromat and 100x/1.4 oil Plan-
Apochromat objectives and a Hamamatsu ORCA-Flash4.0 LT Plus camera. The system is
calibrated and aligned by using 200nm-diameter TetraSpeck microspheres (T7280,
ThermoFisher). Ten to fifty z-stacking images were acquired at 200nm intervals covering
a range from 2-10µm by using ZEN Blue software.
Deconvolution was carried out using Huygens Professional deconvolution software (SVI)
with a measured point-spread-function (PSF) generated by 200nm-diameter TetraSpeck
microspheres. Classical maximum likelihood estimation method with iterations of 40 to
60 and signal-to-noise of 20 to 60 was applied.

**Time-lapse Live-cell microscopy**
Cells were seeded on 2-well or 4-well tissue culture chambers coverglass II (Sarstedt). SiR-DNA (Spirochrome) was added for at least 5hrs prior to live-cell imaging. Images were acquired under a Zeiss AxioObserver Z1 epifluorescence microscopy system equipped with a heating and CO₂ chamber (Digital Pixel) by using 40x/0.6 Plan-Neofluar or 40x/1.3 oil Plan-Apochromat objectives and a Hamamatsu ORCA-Flash4.0 LT Plus camera. For mitotic progression analysis, five to ten z-stacking images with 2µm intervals were taken with the indicated time intervals by using ZEN Blue software. Images were processed using ImageJ software and in-focus z-plane images were manually extracted to make image montages. For imaging of DNA thread formation in live cells, 40x/1.3 oil Plan-Apochromat objective was used to capture eight z-stack images with 800nm intervals and in-focus z-plane images were extracted using ImageJ software.

**Chromosome spread preparation**
Following synchronisation using thymidine, cells were treated with pre-warmed hypertonic solution for 5-10 mins at 37°C (0.075M KCL). The swollen cells were then fixed and washed twice with methanol:acetic (3:1 ratio), before finally being re-suspended in fresh methanol:acetic solution. Chromosome spreads were dropped onto glass slides and either counterstained with Vectashield plus DAPI, or stored at room temperature for forthcoming FISH hybridisation. Colcemid was omitted in all mitotic spread preparations.

**Centromere & telomere Peptide Nucleic Acid (PNA) FISH**
Centromere (CENP-B-FAM; PNABio) & Telomere (Tel-Cy3 PNA FISH kit; DAKO, Agilent) PNA probes were hybridised according to the manufacturer's instructions. Briefly, chromosome spreads were rehydrated in 1X TBS prior to fixation in 3.7% PFA solution. Slides were then washed and pre-treated before dehydration using a gradient ice-cold ethanol wash (70%, 90% and 100%). Slides were air dried and PNA probes were added. Slides were then co-denatured at 80°C for 1 minute and incubated for 2 hours at room temperature. Slides were then washed in FISH Wash solution (Tel-Cy3 PNA FISH kit; DAKO, Agilent) for 5 mins at 65°C following by dehydration using a series of ethanol wash before counterstaining using DAPI Vectashield.

**Multi-colour FISH**
mFISH was performed by using 24XCyte Human Multicolor FISH probe (MetaSystems) according to the manufacturer's instructions. Images were acquired by MetaSystems using a Zeiss AxioObserver Z1 epifluorescence microscopy system with a CoolCube CCD camera and 100x/1.4 oil Plan-Apochromat objective. Multi-colour FISH (mFISH) karyotyping was carried out by using ISIS Imaging software.

**Immunoblotting**
Cells were trypsinized and lysed on ice for 15-20 mins with lysis buffer (50mM Tris pH 7.5, 300mM NaCl, 5mM EDTA, 1% Triton X-100, 1.25 mM DTT, 1mM PMSF and cOmplete™ protease inhibitor cocktail). Protein concentration was quantified using a Bradford assay (Bio-Rad). Immunoblotting (IB) was performed following standard procedures. Primary antibodies used for IB in this study: anti-BLM (Abcam, ab2179, 1:2000), anti-PICH (Abnova; H00054821-B01P, 1:300), anti-GFP (Abcam, ab290, 1:1000) and anti-Ku80 (Abcam, ab80592, 1:10000). All uncropped blot scans are available in the Supplementary excel data file.
**Flow cytometry**

Cells were trypsinised, washed with PBS and fixed with 70% ice-cold ethanol. For cell cycle analysis, cells were washed with PBS and re-suspended in propidium iodide (PI)/RNaseA staining buffer. FACS profiles were then determined and analysed using BD Accuri C6 sampler.

**Kinetochore/centromere foci measurement**

Samples were subjected to pre-extraction in pre-extraction buffer (20mM HEPES pH7.4, 0.5% Triton X-100, 50mM NaCl, 3mM MgCl2, 300mM sucrose) for 10-15sec followed by fixation and immunofluorescent staining as described above. Thirty to fifty z-stacking images with 200nm intervals were acquired and deconvolved using Huygens Professional deconvolution software (SVI). Kinetochore foci on each single z-plane were marked and measured using the ImageJ Plugins detailed below.

**ImageJ measurement of kinetochore foci coordinates, distances and intensities**

Spot Pair Distance Tool: Measures the distance between spots in 2 channels of an image. The tool searches within a focus/box radius, typically +/- 5px, for a local maxima in the two pre-selected analysis channels. The centre-of-mass around each maxima, typically +/- 2px, is computed as the centre of intensity for each channel. Dragging from the clicked point creates a reference direction. The Euclidean distance between the centres is reported, optionally with the signed XY distance and angle relative to the reference direction. Visual guides are overlaid on the image to assist in spot selection and direction orientation. Available in the latest GDSC ImageJ plugins.

Spot Fit Tool: Fits a 2D Gaussian to a spot in an image. The tool searches within a box radius, typically +/- 3px, for a local maxima in the pre-selected analysis channel. A 3x3 smoothing filter is applied before identification of the maxima. A 2D Gaussian function is then fitted to the data using non-linear least-squares fitting and poor fits rejected using a signal-to-noise ratio. The parameters for the fit are reported including the total intensity under the Gaussian function and the local background value. Visual guides are overlaid on the image to show the fitted location. Available in the pre-release GDSC SMLM ImageJ plugins.

**Statistics**

Statistical analysis was performed using GraphPad Prism 7 software by two-tailed unpaired Student’s t-test and two-way ANOVA as per the experimental requirement.

**Recombinant DNA and Transfections**

CENPB (1-158aa) cDNA fragment was PCR amplified from a PLK1 plasmid in which the C-terminal PBD domain was replaced with the first 158 amino acids of CENPB (pQCXIN-Flag-Plk1deltaC-CENPB(1-158)) (a gift from Mark Burkard) and cloned into full length pEGFP-hBLM and pEGFP-hBLM(Q672) plasmids at AgeI site to generate N-terminally tagged CENPB(1-158aa) fusion proteins. Transfections of DNA plasmids were performed using FuGene HD (Promega) according to the manufacturer's guidelines. All plasmids and their sequences are available upon request. Forward primer: (CENPB-For1) 5’-TAAGCAACCGGTATGGGCCGAAGAGCAGACAG-3’; Reverse primer: (CENPB-linker-Rev1)5’-TAAGCAACCGGTCTAGCACTTGCGCCCCAGCACCTTGCTCCACCGGGCGGACTG
Data availability
The authors declare that the data supporting the findings of this study are available within the paper and its supplementary information files. Raw imaging data are available from the corresponding author upon reasonable request.

References
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Author contributions
O.A.J. and K.L.C designed and performed the experiments with help from A.T., T.O. and A.H. O.A.J, A.T., T.O and K.L.C analysed data. K.L.C wrote the manuscript with inputs from all authors.

Declaration of interests
The authors declare no competing interests.
**Figure Legends**

**Figure 1. PLK1 inactivation causes metaphase collapse**  
(a) Experimental outline and time-lapse imaging examples showing the mitotic progression of RPE1 cells under the indicated drug treatment. RPE1 cells were arrested in G1/S by a single thymidine block. Five hours post G1/S-release, the indicated inhibitors were added for another 3 hours, followed by live-cell microscopy (started at t=8hr post G1/S release). Time-lapse imaging was carried out with a 5min interval. (b) Percentages of prophase cells establishing metaphase(-like) alignment and progressing into anaphase under the indicated treatment. 41, 29, 21, 52 and 58 prophase cells were analysed in untreated, monastrol, nocodazole, ProTAME and BI2536 treated conditions. (c) The durations of metaphase(-like) and collapse stages (hrs:mins) of RPE1 cells under the indicated treatment. Note: only cells that established a metaphase(-like) stage were measured. 23, 17 and 19 cells were analysed in untreated, ProTAME and BI2536 treated conditions. DNA was stained by SiR-DNA. Scale bars=5µm.

**Figure 2. Formation of centromere DNA linkages after PLK1 inactivation**  
(a) Experimental outline (top) and representative images showing the immunofluorescent staining of PLK1 in DMSO- and BI2536-treated RPE1 pre-anaphase cells (prometaphase and metaphase). BI2536 induced the formation of a PLK1-decorated threadlike structure (arrows) in the ‘metaphase collapse’ cell. Enlarged region is shown at right. Quantification (bottom) showing the percentage of cells positive for PLK1-coated threads (mean±S.D. is shown; n=3 independent experiments analysing 75 and 224 pre-anaphase cells in DMSO and BI2536 treated conditions, respectively). (b) The experimental setup is same as in (a). PICH co-localises with PLK1 on the threadlike structures induced by BI2536. Quantification (below) showing the percentage of cells positive for PICH-coated threads (mean±S.D. is shown; n=3 independent experiments analysing 120 and 308 pre-anaphase cells in DMSO and BI2356 treated conditions, respectively). (c) Representative images showing the association of the UFB-binding complex (PICH, BLM and RPA70) along the threadlike molecules induced by BI2536. Enlarged regions of both z-projected (below) and single z-planes (right) are shown. Eighty-eight thread structures were examined and all were positive for PICH, BLM and RPA70 staining. Arrows showing regions where the RPA70 staining is strong but with no or weak PICH/BLM signals. Note: BLM was stained by a goat antibody (C-18) (d) RPA32 localises on the thread regions where BLM signal is weak (arrows). Note: BLM was stained by a rabbit antibody (ab2179). (e) DNA threads link between centromeres (arrows).
Enlarged regions of z-projection and single z-planes are shown below. Note: all DNA threads examined (171/171 in 10 cells; 100%) are positive for centromere linkages at either one or both of their termini. (f) Representative images showing cohesed sister chromatids as labelled by TOP2A (arrows) are linked by DNA threads at their centromeres (arrowhead). The enlarged region is shown below. DNA was stained by DAPI. Scale bar=5 µm.

**Figure 3. PLK1 inactivation induces spindle-dependent centromere dislocation**

(a) Experimental outline (top) and representative deconvolved images showing chromosomes isolated from RPE1 cells under the indicated treatment. Chromosomes were hybridised with FISH DNA probes against centromeres (green) and telomeres (red). Left panels: examples of normal chromosome configuration (+nocodazole). Middle panels: examples of BI2536-induced ‘centromere dislocations’ (asterisks; 1, 2, 4 & 5), ‘partial centromere splitting’ (arrowhead; 3), and a centromere DNA thread linking two separate chromosome arms (connecting arrow; 5). Right panels: chromosomes with ‘partial centromere splitting’ (arrowhead) after BI2536 and nocodazole co-treatment. Note: also see Supplementary Figure. 7 for the whole chromosome spread images. (b) Quantification of ‘chromosome arm dislocations’ (left) and ‘partially centromere splitting’ (right) under the indicated inhibitor treatment (n=3 independent experiments analyzing 75 spreads in each condition; the means of each experiment are shown). (c) A diagram depicting the outcomes of chromosome breakage within or outside centromeres. (i) Breakage at centromeres generates both broken arms (100%) positive for CEN FISH signal; (ii) Breakage at pericentric or arm regions generates one of the broken arms (50%) positive for a CEN FISH signal. Quantification (right) of the examined broken chromosome arms with or without centromere FISH signal at their termini (524 broken chromosome arms were scored from 11 separate chromosome spreads showing the highest centromere dislocation frequency). (d) Experimental outline (top) and mFISH karyotyping of RPE1 cells. BI2536 induced chromosome ‘p’- and ‘q’-arm separation. Note: there is a marker ‘M’ chromosome with a translocation of chromosome X and 10 in RPE1 cells. Bar graph (bottom) showing the frequency of ‘centromere dislocations’ among individual chromosomes. Inset graph showing the positive correlation between chromosome length and ‘centromere dislocation’ frequency (23 spreads were analysed). Note: acrocentric chromosomes were not determined and the length of the ‘marker’ chromosome is unknown. (e) Condensin (SMC2) is detected on some PLK1-associated DNA threads (arrows). Scale bar=5 µm. (f) A model of centromere dislocation induced by PLK1 inactivation in a spindle-dependent manner. Spindle-mediated tension causes
decompaction of centromere axis, the formation of centromere DNA threads and whole-chromosome arm separation.

**Figure 4. PLK1 inactivation increases PICH, BLM and RPA foci at K-chromatin**

(a) Increased RPA foci formation at centromeres during mitotic progression after PLK1 inhibition (from prophase to metaphase-like stage). Right: Quantification of the numbers of RPA foci at centromeres in the indicated mitotic stages and treatments (n=3 independent experiments analysing a total of 30 cells in each stage of early, mid prometaphase and metaphase; and of 29 cells in the nocodazole-treated condition; average mean is shown). (b) BI2536 increased the formation of centromeric RPA foci in pre-collapsed metaphase-like cells. Representative images comparing RPA foci at centromeres in DMSO- (left) and BI2536-treated (right) metaphase-like cells. Enlarged images of the selected regions are shown at right. Arrows indicate centromeric RPA foci. Quantification of RPA foci number at centromeres of metaphase (DMSO), and metaphase(-like) (BI2536) cells (n=3 independent experiments analyzing 47 cells per condition). (c) Same as (b) but stained with BLM (n=3 independent experiments analysing 59 and 60 cells in DMSO and BI2536 treated conditions; means of each experiment are shown). (d) Mapping the locations of PICH/BLM/RPA complex at centromeres. Representative images showing the relative locations of PICH (top), BLM (middle) and RPA (bottom) at the centromeres, comparing to the inner kinetochore marker, CENPA. Profile plots of signal intensity accompanies each example. Right: graphs showing the relative position of each protein at both sides of the centromere. (e) A model depicts the localisation of the PICH/BLM/RPA complex at a specific domain of centromeres, named kinetochore-chromatin/K-chromatin. Note: all RPE1 cells analysed were pre-synchronised at G1/S by a single thymidine block. Drugs were added at 6hrs post release. After 2hr treatment, cells were subject to immunofluorescence staining. Scale bars=5µm.

**Figure 5. Loss of kinetochore attachment at centromeres after dislocation**

(a) Representative images showing the kinetochore complex detaches from the core centromere, whilst remaining connected by PICH- or RPA-coated DNA threads (arrows) in BI2536-treated metaphase-like RPE1 cells. Inner and outer kinetochores were labelled by CENPA and NUF2, respectively. (b) Examples showing the majority of centromeres retain two kinetochores in pre-collapsed mitotic populations (prometaphase and metaphase-like cells) after BI2536 treatment. (c) Representative image showing the metaphase-collapse cells losing kinetochore complex at one side of the centromere. Note:
the side without the kinetochore is concomitant with the formation of PICH-associated DNA linkages (arrows). Enlarged images (1 & 2) highlight the loss of CENPA signal at regions of where PICH-decorated DNA linkages form (arrows). (d) Quantification of the numbers of CENPA- and NUF2-labelled kinetochores at centromeres in prometaphase, metaphase-like and collapse stages after BI2536 treatment (n=3 independent experiments analyzing a total of 3020 CENPA-labelled centromeres and 3625 NUF2-labelled centromeres; mean ± S.D. is shown). Note: all RPE1 cells analysed were pre-synchronised at G1/S by single thymidine block. Drugs were added at 6hrs post release. After 2hr treatment, cells were subject to immunofluorescence staining. Scale bars=5µm.

Figure 6. BLM helicase activity triggers centromere disintegration

(a) Western blot showing BLM depletion after RNAi treatment in RPE1 cells. Ku80 is used as a loading control. (b) BLM depletion reduced centromeric RPA foci formation induced by BI2536 in pre-collapsed metaphase(-like) cells. Representative images showing the loss of RPA, but not PICH foci, at centromeres in siBLM cells. Right: quantification of centromeric RPA foci in metaphase(-like) cells (n=3 independent experiments analysing 60 cells per condition; mean ± S.D. is shown). (c) Representative images and quantification of DNA thread formation in wild-type (HAP1) cells, BLM knockout cells (ΔBLM), and ΔBLM HAP1 cells complemented with a wildtype GFP-BLM (WT) and a BLM-helicase mutant (Q672R) protein under BI2536 treatment (n=3 independent experiments analysing a total of 291, 218, 204 and 184 cells in HAP1, ΔBLM, WT and Q672R cell lines; mean ± S.D. is shown) (d) Representative chromosome images of ‘centromere dislocations’ (yellow asterisks) in the indicated HAP1 cells shown in (c). Note: occasional arm breaks (arrow) were observed in ΔBLM cells. Quantification of centromere dislocation is shown below (n=3 independent experiments analysing a total of 60, 60, 51 and 51 spreads in HAP1, ΔBLM, WT and Q672R cell lines; means of each experiment are shown). (e) Centromeric RPA foci formation in ΔBLM HAP1 cells expressing wildtype GFP-BLM (WT) and a BLM helicase-dead mutant (GFP-Q672R) following BI2536 treatment. Representative images showing the lack of RPA foci at centromeres in the GFP-Q672R cells. Right: bar graphs showing the average fluorescence intensities of centromere GFP and RPA foci, respectively, in GFP-BLM and GFP-Q672R cells (mean ± S.E.M. is shown). A scatter plot of RPA foci intensity by GFP foci intensity at centromeres (total numbers of centromere foci analysed: GFP-BLM, n=582; and GFP-Q672, n=481). All RPE1 and HAP1 cells, including their derivatives, were pre-synchronised at G1/S by single thymidine block. Drugs were added at 6hrs post release.
After 2hr treatment, cells were subject to immunofluorescence staining. RNAi treatment of RPE1 cells was performed for 23hrs before G1/S release. Scale bars=5µm.

Figure 7. PICH acts upstream of BLM in centromere disintegration
(a) Experimental outline of RNAi depletion, in combination with thymidine synchronisation and drug treatment. Right: western blot showing PICH protein level in siCTRL and siPICH treatment. Ku80 is used as a loading control. (b) Centromeric DNA thread formation in siCTRL and siPICH cells under BI2536 treatment. Quantification of DNA thread formation in RPE1 cells after siCTRL and siPICH treatment (n=3 independent experiments analysing 315 and 314 cells in siCTRL and siPICH conditions, respectively; mean ± S.D. is shown). (c) Representative images of mitotic spread chromosomes from cells prepared in (a) showing centromere dislocations (asterisks) and partial centromere splitting (arrow). Quantification of centromere dislocation is shown at right (n=3 independent experiments analysing 64 and 61 dislocated chromosome arms in siCTRL and siPICH conditions, respectively; means of each experiment are shown). (d) Reductions of BLM and RPA foci formation at centromeres in siPICH cells after BI2536 treatment. Representative images (left) and quantifications (right) are shown (n=3 independent experiments analysing a total of 60 cells in each treatment; means of each experiment are shown, Scale bars=5µm).

Figure 8. Constitutive PLK1 activity suppresses centromere disintegration
(a) Experimental outline and time-lapse live-cell images of GFP-tagged PLK1 RPE1 cells treated with BI2536 after metaphase establishment. Cells were arrested at metaphase by ProTAME after G1/S release. High-resolution movies were recorded immediately after the addition of BI2536. The formation of DNA threads is revealed by GFP-PLK1 protein (arrows). (b) Experimental outline and quantification of DNA thread formation in metaphase-arrested RPE1 cells. BI2536 was added in ProTAME-arrested metaphase cells for 30 minutes. Centromeric DNA threads were labelled by PICH, BLM and RPA staining. BLM thread counting (n=3 independent experiments analysing a total of 188 and 189 cells in ProTAME and ProTAME+BI2536 conditions, respectively. RPA thread counting (n=3 independent experiments of a total of 174 and 187 cells in each condition; mean ± S.D. is shown). (c) Depletion of PICH or BLM, prolongs the metaphase-(like) stage of RPE1 cells under PLK1 inactivation. Time-lapse microscopy images showing the mitotic progression of RPE1 cells treated with the indicated siRNA oligos, and BI2536. Red bars indicate the metaphase-(like) stage; yellow bars indicate ‘metaphase collapse’. Quantification (right) of the overall duration of metaphase-(like) stage in control, PICH- and BLM-depleted cells,
following BI2536 treatment (n=3 independent experiments analysing 50, 60 and 57 cells in siCTRL, siPICH and siBLM conditions; means of each experiment are shown). Scale bars=5µm.

**Figure 9. A model of centromere disintegration in chromosome misalignment**
Active PLK1 is required to stabilise chromosome biorientation for chromosome segregation. In addition to the existing role for spindle stabilisation, our current study demonstrates that PLK1 also functions to maintain centromere integrity for chromosome alignment. In the absence of PLK1 activity, centromeres are aberrantly targeted by BLM helicase in a PICH-dependent manner. It leads to unlawful, excessive formation of ssDNA, which impairs centromere configuration and weakens its ability to withhold kinetochore complexes. Forces exerted by the bipolar spindle attachment pull out the centromere chromatin, which might trigger further DNA unwinding by the PICH/BLM complex. As a consequence, it decompacts the centromere axis, leading to the formation of centromere DNA threads and whole-chromosome arm separation. Cells therefore fail to maintain chromosome biorientation and result in metaphase collapse with a ‘Fig-8’ like misalignment pattern. Alternatively, if the spindle attachment is destabilised first, it prevents centromere dislocation but this will lead to whole chromosome misalignment, which probably manifests as more like a ‘Polo’ pattern. Therefore, we propose that PLK1 plays a multifactorial role in establishing and maintaining chromosome biorientation by both protection of centromere integration and stabilisation of KT-MT attachment.
Figure 1. Addis Jones et al.

(a) AProTAME (12µM) + Monastrol (100µM) + BI2536 (60nM)

(b) untreated
+Thymidine (18hrs)
+Inhibitors
RPE1
G1/S release
Live-cell imaging

<table>
<thead>
<tr>
<th>% prophase cells</th>
<th>untreated</th>
<th>nocodazole (50ng/ml)</th>
<th>proTAME</th>
<th>BI2536</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% prophase cells</td>
<td>100%</td>
<td>80%</td>
<td>60%</td>
<td>40%</td>
</tr>
</tbody>
</table>

(c) Untreated

- Metaphase duration
- Collapse

Live-cell imaging starts at 8hrs post G1/S release
(hh:mm)
Figure 2. Addis Jones et al.
Figure 3. Addis Jones et al.

**a**

+Thymidine (18hrs) +Inhibitors
RPE1 → G1/S release +Cen & Tel FISH
+Thymidine +Inhibitors
RPE1 → G1/S release +Cen & Tel FISH

+NOC +BI2536 & NOC

1. Centromere
2. Telomere

**b**

\[
\begin{align*}
\text{Number of dislocated chromosome arms} & \quad p = 0.0744 \\
\text{Number of partially splitting centromeres} & \quad p = 0.0321
\end{align*}
\]

- Noc BI2536 BI+Noc
- Noc BI2536 BI+Noc

\[
\begin{align*}
p & \leq 0.0001 \\
p & = 0.0019
\end{align*}
\]

**c**

(i) Centromeric rupture
(ii) Pericentric rupture

CEN FISH signal + Acentric arm

100% chromatin CEN-FISH positive
50% chromatin CEN-FISH positive

% broken arms (per spread)

0 10 20 30 40 50 60 70 80 90 100%

with CEN without CEN

**d**

+Thymidine (18hrs) +Inhibitors
RPE1 → G1/S release +Multi-colour FISH
+Thymidine (18hrs) +Inhibitors
RPE1 → G1/S release +Multi-colour FISH

+Nocodazole +BI2536

**e**

Merged PLK1 SMC2 SMC2 PLK1 DAPI

**f**

“Normal chromosome disjunction”

Sister DNA intertwinemement (ultrafine DNA bridge)

“Centromere dislocation”

Centromeric axis/thread

Anaphase

Metaphase collapse

R2 = 0.5885
p = 0.0002

% broken arms (per spread)

0 10 20 30 40 50 60 70 80 90 100%

with CEN without CEN
Figure 4. Addis Jones et al.
Figure 5. Addis Jones et al.
Figure 6. Addis Jones et al.
Figure 7. Addis Jones et al.
Figure 8. Addis Jones et al.

**ProTAME alone**

**ProTAME \( \Rightarrow +\text{BI2536 at } T=0\text{min} \)**

**Overall metaphase-like duration (mins)**

- ProTAME only
- ProTAME \( \Rightarrow +\text{BI2536 at } T=0\text{min} \)

- ProTAME alone
- ProTAME \( \Rightarrow +\text{BI2536 at } T=0\text{min} \)

**Incremental metaphase-like duration**

- ProTAME only
- ProTAME \( \Rightarrow +\text{BI2536 at } T=0\text{min} \)

- ProTAME alone
- ProTAME \( \Rightarrow +\text{BI2536 at } T=0\text{min} \)

**% pre-anaphase cells have BLM threads**

- ProTAME only
- ProTAME \( \Rightarrow +\text{BI2536 at } T=0\text{min} \)

- ProTAME alone
- ProTAME \( \Rightarrow +\text{BI2536 at } T=0\text{min} \)

**% pre-anaphase cells have RPA threads**

- ProTAME only
- ProTAME \( \Rightarrow +\text{BI2536 at } T=0\text{min} \)

- ProTAME alone
- ProTAME \( \Rightarrow +\text{BI2536 at } T=0\text{min} \)
(i) Destabilized KT-MT attachment

(ii) Misorganisation of spindle attachment

(iii) Spindle-dependent centromere decompaction & DNA thread formation

(iv) Centromere disintegration & whole chromosome arm separation

(iii) Spindle-dependent centromere decompaction & DNA thread formation

(iv) Centromere disintegration & whole chromosome arm separation

Active PLK1

Inactive PLK1

Chromosome biorientation

Chromosome segregation

Centromere disintegration

Spindle destabilisation

Figure 9. Addis Jones et al.
Supplementary Information

PLK1 facilitates chromosome biorientation by suppressing centromere disintegration driven by BLM-mediated unwinding and spindle pulling

Addis Jones et al.
Untreated + Nocodazole (50ng/ml) + Monastrol (100µM)

+ Thymidine (18hrs) t=5hrs

G1/S release

Asyn.

DNA content

+ Thymidine (18hrs)

Metaphase-like

DNA content

+ BI2536

t=5hrs

t=6hrs

t=10hrs

+/- BI2536

Live-cell imaging

DNA content

+/- BI2536

Live-cell imaging

DNA content

Metaphase-like

'Fig-8' shape collapse

Metaphase-like

'Polo' misalignment

DAPI

Pericentrin

GFP-PLK1

PICH

CEN

UFBs are PLK1 positive.

Metaphase-to-Anaphase Early anaphase Late anaphase

Merged DAPI GFP-BLM

Merged α-tubulin RPA70 α-tubulin RPA70 DAPI

64/64 (100%) PICH-stained anaphase UFBs are PLK1 positive.

Supplementary Figure 1. Addis Jones et al.
Supplementary Figure 1. PLK1 inhibition using the small molecule inhibitor BI2536, induces metaphase collapse and the formation of centromeric DNA threads.

(a) Asynchronous RPE1 cells were treated with or without the indicated inhibitors for 18hrs and stained with DAPI. Indicated number of cells analysed and mitotic index are shown. Nuclei were identified by DAPI staining. Scale bar=10µm. (b) Experimental outline for FACS analysis of synchronised RPE1 cells with or without BI2536 treatment. Following 2mM thymidine treatment for 18hrs, samples were collected for FACS analysis at the indicated time points. BI2536 was added to a sample 6hrs post thymidine block release before FACS analysis. (c) Experimental outline and representative images of time-lapse live-cell microscopy of RPE1 cells released from single thymidine block, after BI2536 treatment. Left panels showing an example of three cells entering mitosis and progressing into a metaphase(-like) stage (green arrows), followed by metaphase collapse. Chromosome misalignment is reminiscent of a ‘Figure-8’ pattern (red arrows). Right panels showing a metaphase(-like) cell collapsing into a typical ‘Polo’ misalignment (yellow arrows). DNA was stained by SiR-DNA. (d) Representative images of RPE1 cells following cell synchronisation and treatment as in (b) showing both mitotic arrest of pre-collapse and collapse populations (yellow arrows denote PICH-coated thread-like structures; white arrowheads indicate centrosome positions). (e) EGFP-tagged BLM protein binds to DNA threads (arrows) in Bloom’s syndrome fibroblasts (GM08505), following BI2536 treatment. (f) Experimental outline and time-lapse live-cell images on pre-synchronised RPE1 cells stably expressing GFP-tagged PLK1, highlighting metaphase collapse and pre-anaphase DNA thread formation (arrows), after BI2536 treatment (DNA was stained by SiR-DNA). (g) Pre-synchronised RPE1 cells showing mitotic progression from metaphase to anaphase and the presence of ultrafine-DNA bridge (UFBs) coated by PLK1 and PICH. Enlarged regions highlight UFB formation (arrows). 64/64, 100% of PICH-positive UFBs were positive for PLK1. (h) Representative image showing the distinct localisation pattern of DNA threads (RPA70), compared with microtubules (α-tubulin) in BI2536-treated RPE1 cells. Scale bars=5µm.
Supplementary Figure 2. Addis Jones et al.
Supplementary Figure 2. Shugosin 1 (SGO1) depletion, but not PLK1 inhibition, promotes premature sister-chromatid separation and the subsequent UFB formation.

(a) Experimental work flow of the cell synchronisation and SGO1 RNAi in RPE1 cells. Mitotic spreads were prepared and analysed for premature loss of sister chromatin cohesion. Representative images (top) and DAPI intensity plot profiles (below) indicated the precocious separation of sister-chromatids in Sgo1 depletion cells. (b) Representative images of immunofluorescent staining in SGO1-depleted cells showing the premature formation of PLK1- & PICH-associated UFB structures (yellow arrows). (c) Experimental work flow and representative images of mitotic spreads in untreated and BI2536 treated RPE1 cells. DAPI-intensity plot profiles indicate the existence of two peaks, highlighting that sister-chromatids remain cohesed in BI2536 treatment (0/90, 0% of spreads analysed showed signs of sister-chromatid separation). Scale bars=5µm.
During DNA replication

Plk1 inhibition starts

69±4% of EdU-negative mitotic population displayed PITs

DNA threads are positive in 14% of the mitotic arrested cells (115 mitotic cells analysed)
Supplementary Figure 3. BI2536 induces centromeric DNA threads in post-DNA replication cells.

(a) Experimental workflow showing the labelling of S-phase cells with EdU while BI2536 treatment was carried out. It distinguishes the mitotic population that encounters PLK1 inactivation outside of S-phase. At 5hrs post single thymidine block release, RPE1 cells were treated with the thymidine analogue EdU, together with BI2536. After 3 hrs incubation, click-it chemistry and immunofluorescent staining were carried out. (b) Both EdU+ve and -ve mitotic populations exhibit DNA thread formation. An image (left) showing a mixture of mitotic cells that are either positive (green arrows; +ve), or negative (red arrows; -ve), for EdU signals. Representative images (right) of RPE1 cells displaying DNA thread formation (arrows) in both EdU-positive and negative cells. 69±4% of EdU negative cells are positive for DNA threads (mean± S.D. is shown). (c) Asynchronous GFP-tagged PLK1 RPE1 cells were arrested at G2 using the CDK1 inhibitor, RO-3306, for 16hrs. 30min post G2 release, BI2536 was added for 60mins followed by imaging. Representative image showing the formation of DNA threads bound by the GFP-PLK1 proteins. (d) BI2536 treatment in asynchronous RPE1 cells induced DNA thread formation. Asynchronous RPE1 cells were treated with BI2536 for 1 hour followed by immunofluorescent staining (115 mitotic cells were analysed). Scale bars=5µm.
Supplementary Figure 4. Addis Jones et al.
Supplementary Figure 4. BI2536 induces centromere DNA thread formation in various human non-transformed and tumour-derived cell lines.

(a) Experimental workflow, representative images of different cell lines and their frequencies of DNA thread formation, after BI2536 treatment. Representative images of each cell line displaying the pre-anaphase mitotic populations from an early stage, denoted as pre-collapse (prophase to metaphase-like), and collapse stages (both ‘Fig-8’ and ‘polo’) (70 of 82-6 cells, 80 of 1BR3 cells, 249 of HCT116 cells and 110 of HeLa cells were analysed). Scale bars=5µm. (b) Live-cell time-lapse microscopy showing the percentages of pre-synchronised RPE1 and HeLa cells establish metaphase(-like) stages during BI2536 treatment. Quantification is shown below. n=number of cells analysed. DNA was stained by SiR-DNA.
Supplementary Figure 5. Addis Jones et al.
Supplementary Figure 5. PLK1 inactivation triggers DNA damage response adjacent to centromeres.

(a) DNA damage responses occur at or surrounding centromeric regions following metaphase collapse and the formation of centromeric DNA threads. Representative images display different mitotic phases of RPE1 cells after BI2536 treatment and immuno-stained with PICH, γH2AX and CEN antibodies. Enlarged boxes denote γH2AX signals (arrows) at (peri)centromeric regions, which are either positive for PICH-coated DNA threads, or alternatively strong PICH foci. Quantitation of mitotic populations showing centromeric γH2AX foci (n=3 independent experiments analysing a total of 106, 103 and 96 cells in each stage of prophase/early prometaphase, metaphase collapse (thread +ve) and prometaphase-like (thread -ve); mean ± S.D. is shown).

(b) Untreated mitotic populations display no sign of DNA damage response at centromeres, as demonstrated by an absence of γH2AX foci. Representative images of different mitotic phases of untreated (DMSO) RPE1 cells, immuno-stained with PICH, γH2AX and CEN antibodies. Quantification of mitotic populations showing centromeric γH2AX foci (n=23 and 21 cells analysed in prophase/early prometaphase and prometaphase populations, respectively, from one experiment). Scale bars=5µm.
Supplementary Figure 6. PLK1 inactivation triggers mitotic chromosome fragmentation in a spindle-tension dependent manner.

(a) Experimental outline of cell synchronisation and treatment. (b) Representative images of mitotic chromosomes spreads after treatment with indicated inhibitors. Scale bar=5µm. (c) Quantification of chromosome/chromatin number of the indicated inhibitor treatment (average chromosome numbers is shown below; a total of 90 spreads were scored in each condition from three independent experiments).
Supplementary Figure 7. Addis Jones et al.
Supplementary Figure 7. PLK1 inhibition specifically induces chromosome breakage at centromeres.

(a) Representative deconvolved and raw FISH images of chromosome spreads following treatment with indicated inhibitors (experimental set up as before from supplementary fig. 6a). Centromeres and telomeres were hybridised with FISH DNA probes. (b) Representative example of a BI2536-treated chromosome spread with chromosome breakage at centromeres. Enlarged (1-8; right) regions have been further cropped to allow for contrast enhancement of individual centromere FISH signal detection at each broken chromatin (note: in the absence of increased contrast, the centromere FISH signal can be difficult to see and therefore appear as missing (arrow heads). Scale bar=5µm.
Supplementary Figure 8. Addis Jones et al.
Supplementary Figure 8. Bipolar spindle-tension is required for DNA thread formation induced by PLK1 inactivation

(a) Experimental outline and representative images showing the abolishment of bipolarity, or spindle-tension prevent the DNA thread formation induced by PLK1 inactivation. Co-treatment of either nocodazole (a spindle poison) or monastrol (Eg5 inhibitor) abolishes DNA threads formation. Scale bar=5µm. (b) Quantification of DNA thread formation after the indicated treatment (n=3 independent experiments analysing a total of 601 and 382 cells in BI2536 and BI2536+nocodazole conditions, respectively; n=3 independent experiments analysing a total of 336 and 274 cells in BI2536 and BI2536+monastrol conditions, respectively; mean ± S.D. is shown).
Supplementary Figure 9. Addis Jones et al.
Supplementary Figure 9. PLK1 kinase activity is required to suppress centromeric DNA thread formation and ‘centromere dislocations’.

(a) A diagram depicting the potential off-target effects of BI2536 on centromere DNA thread formation (e.g. PLK2, PLK3 & PLK4 inhibition), and at the same time, demonstrating how PLK1as cells are insensitive to BI2536, whilst providing sensitivity to PLK1 activity via 3-MB-PP1 addition. (b) Experimental outline of both wildtype RPE1 and GFP-tagged RPE1 PLK1as cells for examining DNA thread formation following the treatment of either BI2536 [60nM] or 3-MB-PP1 analogue [1µM]. (c) Representative images display both wildtype RPE1 and GFP-tagged PLK1as RPE1 mitotic cells, showing DNA threads following BI2536 (WT), or 3-MB-PP1 (PLK1as) treatment. (d) Quantification of DNA thread formation after BI2536 or 3-MB-PP1 treatment in parental and GFP-PLK1as RPE1 cells (n=3 independent experiments analysing a total of 224, 168, 148 and 300 cells in the indicated conditions; mean ± S.D. is shown). (e) Experimental outline for both cell synchronisation steps and combined siRNA oligo targeting of PLK1, before immunofluorescent and chromosome spread preparation. Representative images of PLK1-depleted RPE1 cells forming PICH-coated DNA threads (arrows). Enlarged region (below, arrowheads) denotes the presence of PICH protein localisation at the centromere, despite the absence of PLK1. (f) ctFISH analysis of chromosome spreads from control (siCTRL) and PLK1-depleted (siPLK1) RPE1 cells. Quantifications show the numbers of chromatin (left), and ‘centromere dislocations’ (right) (19 and 20 mitotic spreads of siCtrl and siPlk1 treated RPE1 cells were analysed respectively; mean± S.E.M. is shown; scale bars=5µm).
Untreated (metaphase)

Co-cultured RPE1 (WT & GFP-PLK1as)

No. of centromeric PICH foci per metaphase (like)

Intensity of centromeric PICH foci (a.u.)

Supplementary Figure 10. Addis Jones et al.
Supplementary Figure 10. Loss of PLK1 induces aberrant accumulation of PICH, BLM and RPA at kinetochores before metaphase collapse.

(a) Experimental outline of cell synchronisation and drug treatment (left). Representative images of metaphase (untreated) and metaphase-like (BI2536) cells stained with PICH, NUF2 and centromeres. White arrows denote aberrant PICH accumulation at centromeres after BI2536 treatment. Note: PICH was also occasionally observed at the inner-centromeres of untreated cells (yellow arrows) (b) Experimental outline of cell synchronisation and drug treatment on co-cultured wild-type RPE1 cells and RPE1 GFP-PLK1as cells. Representative images showing the induction of centromeric PICH foci after PLK1 inhibition in wild-type RPE1 and GFP-PLK1as cells following the corresponding BI2536 or 3-MB-PP1 treatment. Right panels: enlarged regions showing strong PICH foci in the corresponding treatment. Quantification of PICH foci numbers (c) (18, 22, 20 and 20 cells in the indicated conditions were analysed; mean ± S.D. is shown) and intensities (d) (543, 79, 321 and 676 centromeric PICH foci were measured in the indicated conditions; mean ± S.E.M. is shown). Metaphase(-like) cells were analysed from co-cultures of wild-type RPE1 and PLK1as cells, after treatment with either BI2536 (left) or 3-MB-PP1 (right). (e) Representative images showing co-localisation of PICH, BLM and RPA foci at centromeres induced by BI2536 in metaphase(-like) RPE1 cells. Scale bars=5μm.
Supplementary Figure 11. High-resolution and -precision deconvolution microscopy reveals UFB-binding complex underneath kinetochores.

(a) Images of a 4-color TetraSpeck bead (left) of 200nm diameter in X, Y and Z. Table showing the full width at half maximum (FWHM) (right) of deconvolved TetraSpeck bead images. Scanline plots (below) show the alignments of X, Y and Z positions of a TetraSpeck bead. (b) Representative image of a metaphase(-like) cell treated with BI2536. Image, plot-profile and graph (left to right) demonstrate the relative locations of PICH, compare to NUF2, in East and West poles of the centromere. (c) Representative images of the locations of NUF2 relative to CENPA in metaphase and early anaphase cells. (d) Relative distances of CENPA to NUF2, in East and West poles of centromeres from untreated RPE1 metaphase and early anaphase cells (left two graphs). Relative distances of NUF2 to PICH, CENPA to PICH, CENPA to BLM and CENPA to RPA in East and West poles of the centromere from metaphase(-like) cells after BI2536 treatment (right four graphs) (n shows the numbers of foci analysed from the indicated number of cells; mean distance of each analysed cell is shown). Scale bars=5µm.
Supplementary Figure 12. Addis Jones et al.
Supplementary Figure 12. BLM depletion reduces both centromere DNA thread formation and ‘centromere dislocations’ induced by PLK1 inactivation.

(a) Experimental outline of BLM RNAi, cell synchronisation and treatment in RPE1 cells. (b) Cell-cycle profile plot (top) of DAPI intensity and scatter plot (below) of DAPI intensity against BLM nuclear intensity using ScanR high-content screening microscopy on control (siCTRL) and BLM-depleted (siBLM) samples. Note: BLM expression is cell-cycle regulated and siBLM generates a G2/M population with low BLM nuclear intensity. (c) Representative image gallery of 100 random cell nuclei as from (b), showing BLM nuclear foci in siCTRL and siBLM-treated RPE1 cells. (d) Graph to show the overall percentage of nuclei positive for BLM foci, as from (b) (7462 and 12637 cells from siCTRL and siBLM-treated cells were measured, respectively). (e) Representative images and quantification of pre-anaphase RPE1 cells forming DNA threads in siCTRL and siBLM oligos treatment after BI2536 treatment (n=3 independent experiments analysing 304 and 326 cells in siCTRL and siBLM conditions respectively; mean ± S.D. is shown). (f) Representative chromosome images and quantification of ‘centromere dislocations’ in siCTRL and siBLM oligos treatment after BI2536 treatment (n=3 independent experiments analysing 62 and 60 mitotic spreads in siCTRL and siBLM conditions respectively; mean of each experiment is shown). (g) Representative images showing the reduction of centromeric BLM, but not PICH, foci in metaphase(-like) cells after RNAi treatment as done in (a). Scale bar=5μm.
Supplementary Figure 13. Addis Jones et al.

(a) Schematic diagram of experimental setup.

(i) IF
(ii) Chromosome spreads (ctFISH)
(iii) Western blot

(b) Western blot analysis of HAP1 ΔBLM GFP-BLM and GFP-Q672R under different conditions.

(c) Western blot analysis of HAP1 ΔBLM + GFP-BLM (re-sorted) and GFP-Q672R.

(d) Bar graph showing % cells positive for GFP-BLM/RPA pre-anaphase DNA threads.

**Notes:**
- **GFP-BLM**
- **endo-BLM**
- **Ku80**
- **ΔBLM**
- **GFP-ΔBLM**
- **ΔBLM +**
- **ΔBLM -**
- **HAP1**
- **+Thymidine**
- **+B2536**
- **G1/S release**
- **t=6hrs**
- **t=8hrs**
- **+BI2536**
- **Δ**
- **ns**
Supplementary Figure 13. BLM-helicase activity is responsible for centromeric DNA unwinding during PLK1 inactivation.

(a) Experimental outline for cell synchronisation and BI2536 treatment, in HAP1 cells. (b) Western blot showing BLM protein levels in HAP1, ∆BLM HAP1 cells, and stable cell lines derived from ∆BLM cells, expressing either a wild-type GFP-BLM, or a helicase mutant GFP-Q672R. Antibodies were used to target either BLM (top), or GFP (bottom). Anti-KU80 was used as a loading control. (c) Western blot showing BLM protein levels in the HAP1 stable cell lines expressing GFP-BLM and GFP-Q672R after re-sorting by FACS. Note: the wild-type GFP-tagged BLM (GFP-BLM) expression is lower than the GFP-Q672R mutant. Antibodies were used to target either BLM (top) or GFP (bottom). Anti-KU80 was used for loading control (*ns=non-specific antibody detection). (d) Quantification of centromere DNA thread formation in the FACS re-sorted cells from (c). Note: a low expression of wildtype GFP-BLM (‘low expression’) but not GFP-Q672R (‘high expression’) induced DNA thread formation after BI2536 treatment (n=3 independent experiments analysing a total of 303 and 291 cells of GFP-BLM and GFP-Q672R cell lines, respectively; mean ± S.D. is shown).
Supplementary Figure 14. Mitotic hyperphosphorylation of BLM and PICH is partially dependent on PLK1 activity.

(a) Western blot image showing different migration forms of BLM protein in RPE1 cells after the indicated treatment for 16 hours. (b) same treatment as in (a), but probed with anti-PICH antibody. Ku80 was used as a loading control.
Supplementary Figure 15. Addis Jones et al.
Supplementary Figure 15. Artificial tethering of BLM at the centromere in HeLa cells does not trigger metaphase collapse.

(a) HeLa cells were transiently transfected with a wildtype GFP-BLM or a CENPB(1-158) fused GFP-BLM protein (CENPB-GFP-BLM). Representative images of HeLa cells at various stages of mitosis showing the localisation of GFP-BLM (left panels) and CENPB-GFP-BLM protein (right panels). (b) Representative images of RPA staining in the transfected HeLa mitotic cells described in (a). (c) Time-lapse microscopy images (top) and quantification (bottom) of the transfected HeLa cells progressing in mitosis. DNA was stained by Sir-DNA (15 and 56 transfected prophase cells of GFP-BLM and CENPB-GFP-BLM cell lines were analysed respectively; Scale bars=5µm).