U.PORTO

FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA

2017/2018

Filipe Fernandes de Sousa

Dissecting the role of CENP-E in the maintenance of chromosome alignment

março, 2018

Filipe Fernandes de Sousa

Dissecting the role of CENP-E in the maintenance of chromosome alignment

Mestrado Integrado em Medicina

Área: Medical and Health Sciences

Tipologia: Dissertação

Trabalho efetuado sob a Orientação de:

Doutor Helder José Martins Maiato

E sob a Co-orientação de:

Doutor Bernard Nunes de Almeida Orr

Trabalho organizado de acordo com as normas da revista:

Oncogene

março, 2018

U.PORTO
FMUP FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

Projeto de Opção do 6º ano - DECLARAÇÃO DE INTEGRIDADE

Eu, Filipe Fernandes de Sousa, abaixo assinado, nº mecanográfico 200703251, estudante do 6º ano do Ciclo de Estudos Integrado em Medicina, na Faculdade de Medicina da Universidade do Porto, declaro ter atuado com absoluta integridade na elaboração deste projeto de opção.

Neste sentido, confirmo que **NÃO** incorri em plágio (ato pelo qual um indivíduo, mesmo por omissão, assume a autoria de um determinado trabalho intelectual, ou partes dele). Mais declaro que todas as frases que retirei de trabalhos anteriores pertencentes a outros autores, foram referenciadas, ou redigidas com novas palavras, tendo colocado, neste caso, a citação da fonte bibliográfica.

Faculdade de Medicina da Universidade do Porto, 21/03/2018

Assinatura conforme cartão de identificação:
Filipe Fernandes de Sousa

U.PORTO
FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO

Projecto de Opção do 6º ano – DECLARAÇÃO DE REPRODUÇÃO

NOME

FILIPE FERNANDES DE SOUSA

NÚMERO DE ESTUDANTE	E-MAIL
200703251	FFILIPE89@HOTMAIL.COM

DESIGNAÇÃO DA ÁREA DO PROJECTO

MEDICAL AND HEALTH SCIENCES

TÍTULO DISSERTAÇÃO/MONOGRAFIA (riscar o que não interessa)

DISSECTING THE ROLE OF CENP-E IN THE MAINTENANCE OF CHROMOSOME ALIGNMENT

ORIENTADOR

HELDER JOSÉ MARTINS MAIATO

COORIENTADOR (se aplicável)

BERNARD NUNES DE ALMEIDA ORR

ASSINALE APENAS UMA DAS OPÇÕES:

É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTE TRABALHO APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☐
É AUTORIZADA A REPRODUÇÃO PARCIAL DESTE TRABALHO (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.) APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☐
DE ACORDO COM A LEGISLAÇÃO EM VIGOR, (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.) NÃO É PERMITIDA A REPRODUÇÃO DE QUALQUER PARTE DESTE TRABALHO.	■

Faculdade de Medicina da Universidade do Porto, 21/03/2018

Assinatura conforme cartão de identificação: Filipe Fernandes de Sousa

DISSECTING THE ROLE OF CENP-E IN THE MAINTENANCE OF CHROMOSOME ALIGNMENT

Filipe Sousa[4,5,6], Bernard Orr[7,8], Helder Maiato[1,2,3]

[1] Chromosome Instability & Dynamics Laboratory, Instituto de Biologia Molecular e Celular, Universidade do Porto, Rua Alfredo Allen 208, 4200-135 Porto, Portugal. maiato@i3s.up.pt.

[2] Instituto de Investigação e Inovação em Saúde-i3S, Universidade do Porto, Rua Alfredo Allen 208, 4200-135 Porto, Portugal. maiato@i3s.up.pt.

[3] Cell Division Group, Experimental Biology Unit, Department of Biomedicine, Faculdade de Medicina, Universidade do Porto, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal. maiato@i3s.up.pt.

[4] Chromosome Instability & Dynamics Laboratory, Instituto de Biologia Molecular e Celular, Universidade do Porto, Rua Alfredo Allen 208, 4200-135 Porto, Portugal. filipe.sousa@ibmc.up.pt.

[5] Instituto de Investigação e Inovação em Saúde-i3S, Universidade do Porto, Rua Alfredo Allen 208, 4200-135 Porto, Portugal. filipe.sousa@ibmc.up.pt.

[6] Cell Division Group, Experimental Biology Unit, Department of Biomedicine, Faculdade de Medicina, Universidade do Porto, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal. filipe.sousa@ibmc.up.pt.

[7] Chromosome Instability & Dynamics Laboratory, Instituto de Biologia Molecular e Celular, Universidade do Porto, Rua Alfredo Allen 208, 4200-135 Porto, Portugal.
Bernard.Orr@i3s.up.pt

[8] Instituto de Investigação e Inovação em Saúde-i3S, Universidade do Porto, Rua Alfredo Allen 208, 4200-135 Porto, Portugal.
Bernard.Orr@i3s.up.pt

Abstract

The goal of mitosis is to ensure faithful segregation of genomic content into two identical daughter cells. As cells enter mitosis, the DNA becomes condensed into mitotic chromosomes, which must establish stable attachments to the mitotic spindle. Upon establishing proper kinetochore-microtubule attachments, chromosomes can then be guided to the center of the cell. Importantly, efficient chromosome congression is a prerequisite for faithful chromosome segregation, since errors during this process may lead to aneuploidy and chromosomal instability (CIN). One of the key players involved in chromosome congression is CENP-E, a kinesin-like motor protein that is enriched at kinetochores during mitosis, and whose pharmacological inactivation has shown promising results in clinical trials. Despite mounting evidence demonstrating a role for CENP-E in chromosome congression, the importance of CENP-E motor activity once chromosomes are already aligned, is highly controversial. To this purpose, we took advantage of selective small-molecule inhibitors to achieve temporal control over CENP-E inhibition after chromosomes have completed alignment in both CIN and non-CIN human cell lines. We used three CENP-E inhibitors, PF-2771, GSK923295 and Compound A, which act through distinct mechanisms. Using a high-throughput screening assay, we calculated the optimal doses required for partial and total CENP-E inhibition with these three compounds and then performed high-resolution live cell imaging to directly test CENP-E function in the maintenance of chromosome alignment. We show, using three distinct inhibitors, that CENP-E activity is required for the maintenance of chromosome alignment in a condition of prolonged metaphase. Interestingly, the formation of chromosomes at poles occurs more rapidly in GSK923295-treated cells, suggesting that inhibiting CENP-E with this compound may lead to undesired microtubule-associated effects. We find that relative sensitivity to CENP-E inhibitors is proportional for PF-2771 and GSK923295 when comparing CIN and non-CIN cell lines. However, diploid cells are less sensitive to CENP-E inhibition using

Compound A, suggesting that this compound may be of higher therapeutic value for the treatment of cancer. Taken together, these results offer insight for our understanding of the therapeutic value of CENP-E inhibitors currently tested in clinical trials.

Keywords: CENP-E; Congression; Chromosome alignment; Mitosis; CIN;

Introduction

An effective mitosis ensures the faithful segregation of genomic content into two identical daughter cells. Precisely at the onset of mitosis, characterized by chromosome condensation and nuclear envelope breakdown, dispersed mitotic chromosomes initiate directed movements towards the cell equator. This stochastic motion defines the mitotic stage of prometaphase, whose goal is to promote the initial attachment between chromosomes and kinetochores, essential for chromosome congression (1). One of the key players involved in chromosome congression is CENP- E, a kinesin-like plus -end-directed motor protein (kinesin-7) that associates with the kinetochore fibrous corona and is strongly enriched at kinetochores during prometaphase (2). A role for CENP-E in chromosome congression was established after live-cell imaging of human cells depleted of CENP-E function by antibody microinjection (3-5). While several chromosomes were able to align after perturbation of CENP-E, others remained in close proximity with spindle poles. Subsequently, only peripheral mono-oriented chromosomes were shown to congress in a CENP-E dependent manner by laterally sliding their unattached kinetochores to neighboring mature kinetochore fibers (6, 7). This model predicted that CENP-E guides misaligned chromosomes towards the cell equator. However, some reports have challenged the idea that CENP-E function is restricted to alignment of chromosomes, raising the intriguing possibility that CENP-E function is required to maintain bi-oriented chromosomes at the equator after completing congression (8). Addressing this question has proven to be challenging since most strategies used to impair protein function rely on RNAi or genome editing techniques, which offer no temporal control over protein function. However, the development of small molecule inhibitors has provided a powerful tool that allows precise temporal control over protein function. Indeed, partial CENP-E inhibition using GSK923295 was shown to result in the accumulation of mono-oriented chromosomes and consequently caused a mitotic delay (9). GSK923295 was also used to propose that CENP-E is motor active at

the kinetochores of congressed chromosomes, thus facilitating the dynamics of microtubule plus ends and kinetochores (8).

To date, the most widely used CENP-E inhibitor is GSK923295, an allosteric small-molecule inhibitor that targets CENP-E motor activity and exhibits potent antitumor activity in several preclinical models of human tumor xenografts (10). The GSK923295 inhibitor binding site was mapped to a region similar to that bound by loop-5 inhibitors of the kinesin KSP/Eg5. However, unlike these KSP inhibitors that block release of ADP and destabilize motor-microtubule interaction, GSK923295 inhibited the release of inorganic phosphate resulting in a conformational state with dramatically enhanced affinity for microtubules causing CENP-E to be bound to microtubules in a rigor-like state (10). Similar to GSK923295, a novel CENP-E inhibitor named PF-2771 was generated to study the effects of CENP-E depletion in human basal breast cancer cell lines (11). Importantly, while PF-2771 is noncompetitive with ATP (it acts independently of substrate loading), GSK923295 is uncompetitive with both ATP and microtubules (it requires both substrates to be loaded to perform its function). More recently, a novel small-molecule inhibitor of CENP-E, Compound-A (Cmpd-A) was developed to specifically inhibit CENP-E function independently of its binding to microtubules (12). Unlike GSK923295 and PF-2771, Cmpd-A inhibits the ATPase activity of the CENP-E motor domain, acting as a time-dependent inhibitor with an ATP-competitive-like behavior. Cmpd-A treatment also causes chromosome misalignment and chromosomes at poles, leading to prolonged mitotic arrest, consistent with other reports where CENP-E function was suppressed. By targeting the L5 binding site at 3 different amino acid residues, CENP-E inhibitory activity was increased by fine-tuned chemical modifications (13, 14). Here, we take advantage of the availability of next generation inhibitors as well as state-of-the-art live cell imaging to demonstrate that CENP-E partial inhibition is sufficient to cause misalignment after bi-orientation in a condition of prolonged metaphase. The extent of the misalignment was independent of the inhibitor of choice and was frequently followed by the formation of

chromosomes at poles in a dose-dependent manner. We find that GSK923295-treated cells showed a more rapid accumulation of chromosomes at poles, suggesting that microtubule-associated phenotypes induced by GSK923295 treatment may account for these differences. We discuss the implications this may have for normal metaphase durations, and the possible therapeutic advantage of Cmpd-A over GSK923295 and PF-2771.

Results

Assessing the sensitivity to chemical inhibitors or pharmacological treatments requires a careful approach to minimize unspecific off-target effects that are concentration dependent (15). In order to establish the optimal doses required for partial and full CENP-E inhibition using each compound, we used an in-house high-throughput screening platform coupled with an automated phenotype imaging analysis (CellProfiler) to quantify mitotic phenotypes in living cells. This automated system of image acquisition, processing and quantification provides a rapid, robust and unbiased phenotypic analysis that can be used with multiple small-molecule inhibitors and cell lines (Fig. 1A and 1B, Supp. Fig. 1A-D). Since peripheral mono-oriented chromosomes rely on CENP-E for efficient congression (7), CENP-E inhibition can reliably be determined by the persisting presence of chromosomes at poles (Supp. Fig. 1C). HeLa, U20S and RPE1 cells were treated with increasing concentrations of CENP-E inhibitors for 3 hours and their phenotype was assessed every hour (Fig. 1C-E, Supp. Fig. 2A and 2B). We find that CENP-E inhibition using all compounds can generate chromosomes at poles in both diploid (RPE1) and CIN (HeLa and U2OS) cell lines, at comparable concentrations (Fig. 1C-E, Supp. Fig. 2A and 2B). We calculated the concentrations for partial inhibition as the lowest concentration required to observe an effect and full inhibition was defined as the lowest concentration required to exert maximal effect in terms of chromosomes at poles (beginning of plateau). Upon reaching the plateau, the percentage of cells with chromosomes at poles was comparable for each compound suggesting that CENP-E can be efficiently inhibited through three distinct mechanisms (Fig. 1F). Nevertheless, this percentage was lower in U2OS and RPE1 cells (Fig. S2A and 2B). Moreover, we found no increase in apoptosis/cell debris within 3 hours of treatment with CENP-E inhibitors (data not shown). Our data suggest that CENP-E inhibition results in the formation of chromosomes at poles, independent of the molecular mechanism used to inhibit CENP-E function.

Using the optimal doses that partially and fully inhibit CENP-E function calculated from the high-throughput screen, we used high-resolution live-cell imaging to determine whether CENP-E motor activity is required for the maintenance of chromosome alignment (Fig. 3A). Using the proteasome inhibitor MG-132, HeLa cells were arrested at metaphase with fully aligned chromosomes, then treated with CENP-E inhibitors and imaged every 5 minutes for 3 hours (Fig. 3A). We find that CENP-E inhibition causes general chromosome misalignment accompanied by chromosomes at poles in all cell lines tested, regardless of the inhibitor used (Figs 2B-E; S3). We also observed clear cohesion fatigue that was specific to CIN cells and was exacerbated by treatment with CENP-E inhibitors (Fig S6). Our live cell imaging setup allows us to clearly distinguish between loss of alignment due to cohesion fatigue, where single chromatids start to "peel-off" from the metaphase plate, and a morphology of paired chromatids arranged as chromosomes at poles, consistently observed after CENP-E inhibition (Fig. S7A, C). Nevertheless, we find that the percentage of cells with chromosomes at poles induced by treatment with CENP-E inhibitors is dose-dependent (Fig. 2B-E). This was also observed in RPE1 and U2OS cells (Fig. S3). Concurrently, we also observe a dose-dependent effect on the percentage of cells that remain aligned throughout the experiment, consistent for all cell lines analyzed (Fig. S4). Direct evidence demonstrating that CENP-E inhibitors were actively disrupting CENP-E function, was obtained from neighboring cells that entered mitosis in the presence of CENP-E inhibitors. As expected, these cells presented several chromosomes at poles immediately after nuclear envelope break down and arrested in mitosis with chromosomes at poles (Fig. S5). When we restrict our analysis of the percentage of cells with chromosomes at poles to the first hour of imaging of these artificial metaphases, we notice that there is a stronger phenotype induced by GSK923295 compared with PF-2771 and Cmpd-A, at full inhibition (Fig. 2B-E; S3). At this time-point, events of cohesion fatigue or other epiphenomena related to drug toxicity or metaphase arrest are less likely to take place (16). During the course of the experiment, we also quantified the frequency of terminal events that dictated the

exclusion of these cells from the analysis of chromosome alignment from that time point onwards (Fig. S6). Terminal events included cells entering anaphase in the presence of MG-132 without chromosome decondensation (Fig. S7B), cohesion fatigue, mitotic slippage and cells that moved out of the field of view or out of focus. We find that cohesion fatigue was highly prevalent in U2OS cells but rarely observed in other cell types. Importantly, this effect also appeared to be potentiated by the presence of CENP-E inhibitors.

Our live cell imaging assay also allows us to determine how often chromosomes re-align or generate chromosomes at poles, after an initial misalignment event (Fig. S8). Although there is some variability, results suggest that after an initial misalignment event, CENP-E is required for chromosome re-alignment. Indeed, CENP-E disruption often leads to chromosomes at poles in a dose-dependent manner, while this rarely occurs in HeLa and U2OS cells upon DMSO treatment.

Next, we analyzed cellular sensitivity to CENP-E inhibitors to understand whether this correlates with the overall effects on chromosome alignment observed using our live cell imaging assay. For that purpose, we used a standard Resazurin Cell Viability assay in which cells were incubated for 72 hours with each CENP-E inhibitor and the IC50s were subsequently calculated (Fig. 3). We find that HeLa cells are the most sensitive to all inhibitors tested and that U2OS cells are generally less sensitive to all compounds when compared to HeLa cells (Fig. 3A-C). Surprisingly, RPE1 cells are 2,1-fold less sensitive to Cmpd-A but are equally sensitive to GSK923295 and PF-2771 treatments suggesting that Cmpd-A has unique properties that potentiate its effect more specifically in CIN cell lines.

Discussion

Microtubule-targeting chemotherapeutics

Modern day chemotherapy begun with the discovery of nitrogen mustard's toxic effects on haematopoietic cells and was first administered to lymphoma patients with marked improvements in tumor regression. However, remissions turned out to be brief and incomplete, prompting the discovery and development of other chemotherapeutic agents (17). Chemotherapeutics include alkylators, antimetabolites, platinum agents and natural products. Spindle poisons are plant-derived natural products that target tubulin, the cytoskeletal protein that polymerizes to form microtubules that participate in a plethora of cellular functions. These compounds are broadly defined as anti-mitotics and can be either microtubule destabilizers (i.e. vinca alkaloids, epothilones and eribulin) or stabilizers (i.e. taxanes). Since microtubules are essential for cellular homeostasis, these agents often cause serious side effects such as neurotoxicity and neutropenia and/or may lead to the acquisition of resistance (18-20). CENP-E is a kinesin motor protein expressed predominantly in mitosis (and G2) (5) and its loss of function causes chromosome misalignment and apoptosis due to a prolonged mitotic arrest (21, 22). Although CENP-E requires microtubules to exert its functions, it slowly accumulates in G1, reaching a peak in G2-M phase due to an increased mRNA level, and is ultimately degraded in the end of mitosis (23). Thus far, no specific function was attributed to CENP-E in interphase and therefore, CENP-E inhibition represents a new class of anti-mitotic therapeutics with the potential to reduce microtubule-associated side-effects.

CENP-E as a molecularly-targeted anti-cancer drug

Several preclinical studies have evidenced the efficacy of CENP-E inhibitors with regards to their anti-tumor activity. PF-2771 treatment induced tumor regression in a patient derived basal-like triple negative cancer xenograft tumor model (11). GSK923295

showed a dose-dependent antitumor activity against mice bearing xenografts of the Colo205 colon tumor-cell line as well as against a panel of 212 tumor cell lines (10). These promising results led to the first Phase I clinical trials using GSK923295 to treat human subjects with refractory cancer that do not respond to standard therapy. Notably, anemia and fatigue were described as the only adverse events, with a very low occurrence of neutropenia, typically very rare for a bona-fide antimitotic drug. Despite the promising results, further studies are required to determine the best administration schedules/techniques to reach optimal plasma concentrations that ensure specific CENP-E inhibition (24). More recently, Compound A (Cmpd-A), a novel small-molecule inhibitor of CENP-E was described and shown to exhibit an antiproliferative effect in 14 cancer cell lines. Surprisingly, the diploid MRC5 cell line demonstrated reduced sensitivity to CENP-E inhibition, suggesting that CENP-E inhibition using Cmpd-A may target a specific form or domain in CENP-E that is altered in cancer cells. In agreement, the authors found no correlation between CENP-E expression and the anti-proliferative effects of Cmpd-A in the cell lines analyzed (12). In our study we used RPE1 cells as a diploid cell line and found that they are 2,1-fold less sensitive to Compound A but are equally sensitive to GSK923295 and PF-2771. In contrast, chromosomally unstable U2OS and HeLa cells showed a comparable sensitivity between all the inhibitors. However, this effect is not simply explained by the efficiency of mitotic arrest since our live cell imaging experiments show that PF-2771 and GSK923295 treatments were able to induce chromosomes at poles with equal or even higher efficiency in the diploid cell line. These data suggest that either Cmpd-A off-target effects are more evident in CIN cells (CENP-E-independent), the diploid cell line RPE1 has higher drug efflux pump activity and can efficiently pump out specific drug structures, or alternatively, Cmpd-A may target CENP-E isoforms that are more commonly observed in CIN cell lines. These hypotheses could be addressed by performing cell viability assays in the presence of inhibitors of the p-glycoprotein, such as verapamil, and by sequencing CENP-E to address the presence of mutations that may confer resistance.

CENP-E as a tethered motor between microtubules and kinetochores

Recent models have proposed that CENP-E plays an important role in facilitating the association between kinetochores and dynamic microtubule ends as a tether motor (8). In order to investigate the contribution of CENP-E to link microtubule plus ends to kinetochores on congressed chromosomes the authors used GSK923295 (locks CENP-E in a microtubule-bound, inactive state) at a concentration 4-fold higher than our optimal dose used for full CENP-E inhibition calculated in our titration experiment. Since chromosomes moved towards the pole, the authors concluded that CENP-E continues active at kinetochores of aligned chromosomes, but at these concentrations GSK923295 treatment may be indirectly affecting kinetochore-microtubule dynamics. In normal cells, average metaphase durations in HeLa, hTERT-RPE1 and U2OS cell lines are 16, 9 and 11 minutes respectively (25). Here we show that in metaphase arrested cells, approximately 30-60 minutes after CENP-E inhibition, misalignment and formation of chromosomes at poles is frequently observed with all compounds tested. However, we show that the formation of chromosomes at poles occurs significantly faster in GSK923295-treated cells, and this appears to be specific to CIN cells. Notably, PF-2771 and Compound A act independently of microtubules while GSK923295 provides a gain of function CENP-E phenotype since it causes a rigor like state in a microtubule-bound state. Under these conditions, it is probable that chromosomes are being stripped off the metaphase plate more frequently due to spindle flux. Although we are working with artificial metaphase extensions, our results demonstrate that CENP-E activity is required for the maintenance of chromosome alignment during a prolonged metaphase. However, whether these results reflect CENP-E function in the context of chromosome alignment in normal physiological conditions, remains unknown.

Using state-of-art live cell microscopy we were able to follow cells through a continued mitotic arrest induced by proteasome inhibition. It is worth noting that the proteasome inhibitor MG-132 was never washed-out during our experimental setting,

which bears important implications for our interpretation of the results. Indeed, recent studies have suggested that a prolonged metaphase arrest may lead to cohesion fatigue, a condition where sister chromatid cohesion is lost but the cell is arrested in mitosis with high levels of Cyclin B1 and separated chromatids (16). Particularly in transformed cells with numerous chromosomes, it may become difficult to distinguish between cohesion fatigue (where individual chromatids are pulled to poles) and whole chromosomes at poles, particularly when looking at static images of cells (i.e., immunofluorescence or time-lapse imaging with low temporal resolution) (26). However, a time-lapse recording with high temporal resolution allows us to follow the dynamics of chromosome and spindle movements, critial for making the distinction between cohesion fatigue and chromosomes at poles (See Figure S7C). Indeed, the striking similarity between the phenotypes of chromosomes and poles and cohesion fatigue incites for a systematic re-evaluation of proteins formerly associated with chromosome alignment using state-of-the-art live cell imaging techniques. Our experimental setup permits us to clearly identify the most dramatic cases of cohesion fatigue on the basis of chromosome morphology, and on the dynamics of chromosome movement and spindle rotation, but the use of a kinetochore marker would provide unequivocal evidence on this subject. Moreover, a proteasome-induced 3-hour mitotic arrest may cause secondary phenotypes that warrant a cautious approach when evaluating the formation of chromosomes at poles, particularly in conditions of prolonged metaphase arrest.

Under the combined effect of MG-132 and CENP-E inhibition, we find that cohesion fatigue is more prevalent in CIN cells, particularly U2OS, and was rarely observed in RPE1 cells. This suggests that CIN cells may be inherently more susceptible to cohesion fatigue, which is in accordance with results observed using a proteasome inhibitor alone (22). And this rationale further validated the usefulness of Bortezomib (27), a proteasome inhibitor approved for multiple myeloma and refractory mantle cell lymphoma with other new compounds currently at preclinical stage testing (28-30).

However, we show that dual inhibition of CENP-E and proteasome activity causes deleterious effects that are exacerbated in CIN cells, thus providing preclinical data in support of a combination therapy regime. Associating CENP-E inhibitors with bortezomib may be another suitable therapeutic strategy in the future, to overcome limitations of individual pharmacokinetics and pharmacodynamics or resistance in drug administration schedules.

Materials and Methods

Cell lines

HeLa, U2OS (kindly provided by M. Barisic) and hTERT-RPE1 cell lines stably expressing H2B-GFP and mCherry-α-Tubulin were used. All cell lines were grown in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Life Technologies) supplemented with 10% fetal bovine serum (FBS; Gibco Life Technologies) at 37°C in a humidified atmosphere with 5% CO_2.

Drugs/compounds used

The CENP-E Inhibitors - GSK923295 (Selleckchem), PF-2771 (Medchem Express) and Compound A (Takeda) - and the proteasome inhibitor MG-132 (Calbiochem) were originally dissolved in DMSO (Sigma-Aldrich) and freshly diluted in DMEM medium without phenol red immediately before live cell imaging. Total volume DMSO, either used as control or drug dilution, added to the culture medium never exceeded 1:1000 v/v.

Microscopy

The titration experiment was performed in the InCell Analyser 2000, equipped with a sCMOS camera, and provided with two laser lines (488 nm and 561 nm). HeLa, U2OS and hTERT-RPE1 (all stably expressing H2B-GFP/mCherry-α-tubulin) cells were kept in a heated chamber (37 °C), with controlled CO_2 conditions. Using a 20x objective (0.45 NA), images were collected every hour after CENP-E inhibition, up to 3 hours. Image analysis was performed with CellProfiler software.

After determining the optimal dose for inhibiting CENP-E function, live-cell confocal microscopy was performed. HeLa, U2OS and hTERT-RPE1 (all stably expressing H2B-GFP/mCherry-α-tubulin) cells were plated onto 6 well-plates with pre-cut coverslips 24 hours prior to the assay. To promote a cell cycle arrest at metaphase, 3 μM of MG-132 prepared in DMEM without phenol red was added to the cells approximately 30 minutes

before live cell imaging. After the first frame of imaging (5min), DMSO or the selected concentration of inhibitor was added and cells were followed through time-lapse confocal microscopy. Time-lapse imaging was performed in a heated chamber (37°C) using a 100x 1.4 NA Plan-Apochromatic differential interference contrast objective mounted on an inverted microscope (Ti; Nikon) equipped with a CSU-X1 spinning-disk confocal head (Yokogawa Corporation of America) and with two laser lines (488 nm and 561 nm). [MM1] Images were detected with an iXonEM+ EM-CCD camera (Andor Technology). Nine 2µm lengh z-planes for each channel covering the entire volume of the mitotic spindle were collected every 5 min, for up to 180 minutes. Image and video processing was performed using Image J and Nikon NIS viewer, respectively.

CellProfiler pipelines and settings

CellProfiler's object identification modules contain a variety of published and tested algorithms for identifying cells based on fluorescence (31). We firsly defined the primary objects as nuclei, through the H2B-GFP signal, and then the secondary objects as cell size and shape, through the Tubulin-mCherry signal. After rendering this segmentation, a gallery of cells was manually classified into four categories for each cell line: interphase, mitotic (excluding chromosomes at poles), chromosomes at poles and apoptosis/cell debris (Fig. S1A-D). This gallery was used as a training set that served the automated classification of the Cell Profiler software.

IC50 and cell proliferation assay

HeLa (1500 cells/well), RPE1 (8000 cells/well) and U2OS (3000 cells/well) cells were seeded on a sterile 96-well plate (TC-Plate 96 well, Cell+, F, Sarstedt) and let adhere for 24h. The cells were treated with all CENP-E inhibitors freshly dissolved in DMEM media for 72 hours at the following concentrations: 8 nM, 16 nM, 31 nM, 62.5 nM, 125 nM, 250 nM, 500 nM for PF-2771 and Compound A and 4 nM, 8 nM, 16 nM, 31 nM, 62.5 nM, 125 nM, 250 nM for GSK923295. The cells were then washed with 1X PBS and incubated

with 0,1% Resazurin (Sigma-Aldrich) dissolved in DMEM for 4 hours. The supernatant was transferred to a new 96-well plate and its resorufin flourescence was measured using a microplate spectrofluorometer (Synergy MX, Biotek) with the following settings - Excitation wavelengh: 530±9 nm, Emission wavelengh: 590±9 nm.

Acknowledgments

We would like to acknowledge AF Maia for his help with the InCell Analyzer 2000 and initial assessment of CellProfiler software.

References

1. Maiato H, Gomes AM, Sousa F, Barisic M. Mechanisms of Chromosome Congression during Mitosis. Biology (Basel). 2017;6(1).

2. Yen TJ, Compton DA, Wise D, Zinkowski RP, Brinkley BR, Earnshaw WC, et al. CENP-E, a novel human centromere-associated protein required for progression from metaphase to anaphase. EMBO J. 1991;10(5):1245-54.

3. Wood KW, Sakowicz R, Goldstein LS, Cleveland DW. CENP-E is a plus end-directed kinetochore motor required for metaphase chromosome alignment. Cell. 1997;91(3):357-66.

4. Yao X, Anderson KL, Cleveland DW. The microtubule-dependent motor centromere-associated protein E (CENP-E) is an integral component of kinetochore corona fibers that link centromeres to spindle microtubules. J Cell Biol. 1997;139(2):435-47.

5. Yen TJ, Li G, Schaar BT, Szilak I, Cleveland DW. CENP-E is a putative kinetochore motor that accumulates just before mitosis. Nature. 1992;359(6395):536-9.

6. Kapoor TM, Lampson MA, Hergert P, Cameron L, Cimini D, Salmon ED, et al. Chromosomes can congress to the metaphase plate before biorientation. Science. 2006;311(5759):388-91.

7. Barisic M, Aguiar P, Geley S, Maiato H. Kinetochore motors drive congression of peripheral polar chromosomes by overcoming random arm-ejection forces. Nat Cell Biol. 2014;16(12):1249-56.

8. Gudimchuk N, Vitre B, Kim Y, Kiyatkin A, Cleveland DW, Ataullakhanov FI, et al. Kinetochore kinesin CENP-E is a processive bi-directional tracker of dynamic microtubule tips. Nat Cell Biol. 2013;15(9):1079-88.

9. Magidson V, Paul R, Yang N, Ault JG, O'Connell CB, Tikhonenko I, et al. Adaptive changes in the kinetochore architecture facilitate proper spindle assembly. Nat Cell Biol. 2015;17(9):1134-44.

10. Wood KW, Lad L, Luo L, Qian X, Knight SD, Nevins N, et al. Antitumor activity of an allosteric inhibitor of centromere-associated protein-E. Proc Natl Acad Sci U S A. 2010;107(13):5839-44.

11. Kung PP, Martinez R, Zhu Z, Zager M, Blasina A, Rymer I, et al. Chemogenetic evaluation of the mitotic kinesin CENP-E reveals a critical role in triple-negative breast cancer. Mol Cancer Ther. 2014;13(8):2104-15.

12. Ohashi A, Ohori M, Iwai K, Nambu T, Miyamoto M, Kawamoto T, et al. A Novel Time-Dependent CENP-E Inhibitor with Potent Antitumor Activity. PLoS One. 2015;10(12):e0144675.

13. Hirayama T, Okaniwa M, Banno H, Kakei H, Ohashi A, Ohori M, et al. Design and synthesis of fused bicyclic inhibitors targeting the L5 loop site of centromere-associated protein E. Bioorg Med Chem Lett. 2016;26(17):4296-300.

14. Hirayama T, Okaniwa M, Imada T, Ohashi A, Ohori M, Iwai K, et al. Synthetic studies of centromere-associated protein-E (CENP-E) inhibitors: 1.Exploration of fused bicyclic core scaffolds using electrostatic potential map. Bioorg Med Chem. 2013;21(17):5488-502.

15. Asteriti IA, Di Cesare E, De Mattia F, Hilsenstein V, Neumann B, Cundari E, et al. The Aurora-A inhibitor MLN8237 affects multiple mitotic processes and induces dose-dependent mitotic abnormalities and aneuploidy. Oncotarget. 2014;5(15):6229-42.

16. Daum JR, Potapova TA, Sivakumar S, Daniel JJ, Flynn JN, Rankin S, et al. Cohesion fatigue induces chromatid separation in cells delayed at metaphase. Curr Biol. 2011;21(12):1018-24.

17. DeVita VT, Jr., Chu E. A history of cancer chemotherapy. Cancer Res. 2008;68(21):8643-53.

18. Gotaskie GE, Andreassi BF. Paclitaxel: a new antimitotic chemotherapeutic agent. Cancer Pract. 1994;2(1):27-33.

19. Kavallaris M. Microtubules and resistance to tubulin-binding agents. Nat Rev Cancer. 2010;10(3):194-204.

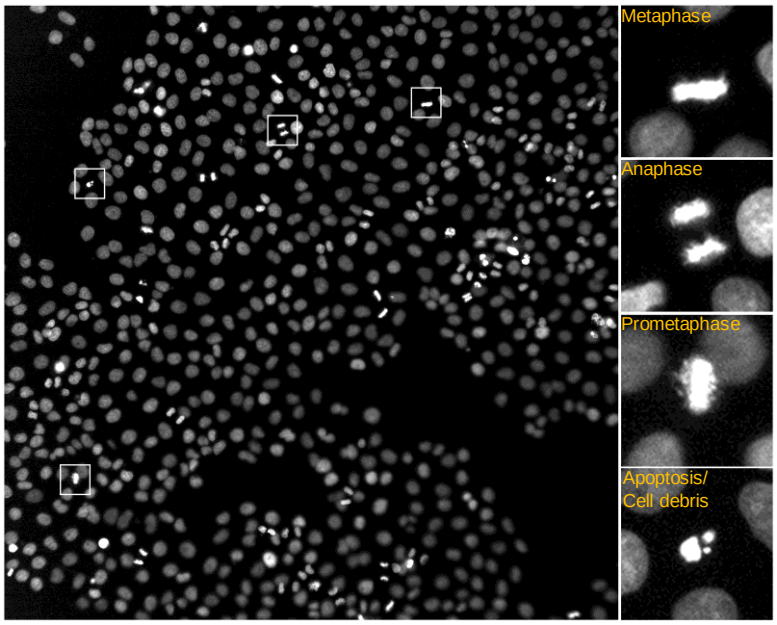
435 20. Zhou XJ, Rahmani R. Preclinical and clinical pharmacology of vinca alkaloids.
436 Drugs. 1992;44 Suppl 4:1-16; discussion 66-9.
437 21. Ohashi A, Ohori M, Iwai K, Nakayama Y, Nambu T, Morishita D, et al. Aneuploidy
438 generates proteotoxic stress and DNA damage concurrently with p53-mediated post-
439 mitotic apoptosis in SAC-impaired cells. Nat Commun. 2015;6:7668.
440 22. Stevens D, Gassmann R, Oegema K, Desai A. Uncoordinated loss of chromatid
441 cohesion is a common outcome of extended metaphase arrest. PLoS One.
442 2011;6(8):e22969.
443 23. Brown KD, Coulson RM, Yen TJ, Cleveland DW. Cyclin-like accumulation and
444 loss of the putative kinetochore motor CENP-E results from coupling continuous
445 synthesis with specific degradation at the end of mitosis. J Cell Biol. 1994;125(6):1303-
446 12.
447 24. Chung V, Heath EI, Schelman WR, Johnson BM, Kirby LC, Lynch KM, et al. First-
448 time-in-human study of GSK923295, a novel antimitotic inhibitor of centromere-
449 associated protein E (CENP-E), in patients with refractory cancer. Cancer Chemother
450 Pharmacol. 2012;69(3):733-41.
451 25. Pereira AJ, Maiato H. Maturation of the kinetochore-microtubule interface and the
452 meaning of metaphase. Chromosome Res. 2012;20(5):563-77.
453 26. Gorbsky GJ. Cohesion fatigue. Curr Biol. 2013;23(22):R986-8.
454 27. Adams J. The proteasome: a suitable antineoplastic target. Nat Rev Cancer.
455 2004;4(5):349-60.
456 28. Chauhan D, Singh AV, Aujay M, Kirk CJ, Bandi M, Ciccarelli B, et al. A novel
457 orally active proteasome inhibitor ONX 0912 triggers in vitro and in vivo cytotoxicity in
458 multiple myeloma. Blood. 2010;116(23):4906-15.
459 29. Einsele H. Bortezomib. Recent Results Cancer Res. 2010;184:173-87.
460 30. Mato AR, Feldman T, Goy A. Proteasome inhibition and combination therapy for
461 non-Hodgkin's lymphoma: from bench to bedside. Oncologist. 2012;17(5):694-707.

462 31. Carpenter AE, Jones TR, Lamprecht MR, Clarke C, Kang IH, Friman O, et al.
463 CellProfiler: image analysis software for identifying and quantifying cell phenotypes.
464 Genome Biol. 2006;7(10):R100.

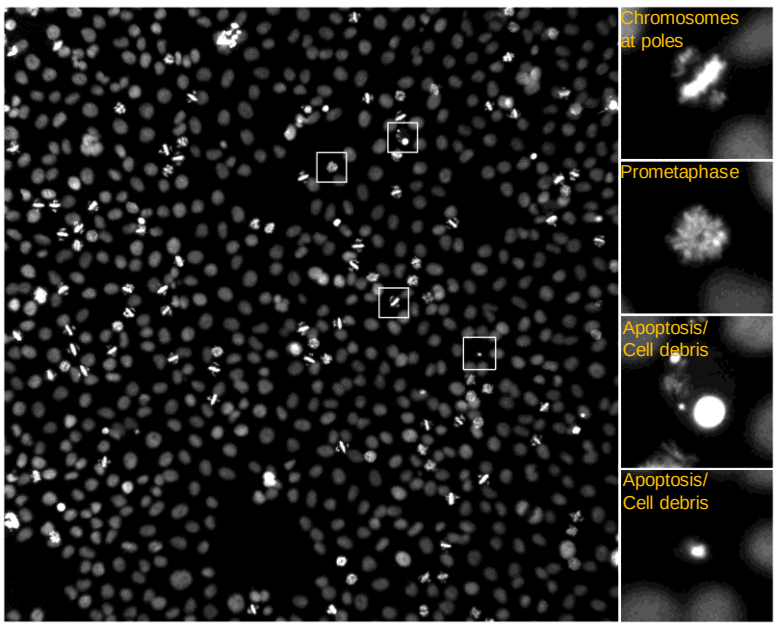
465

466

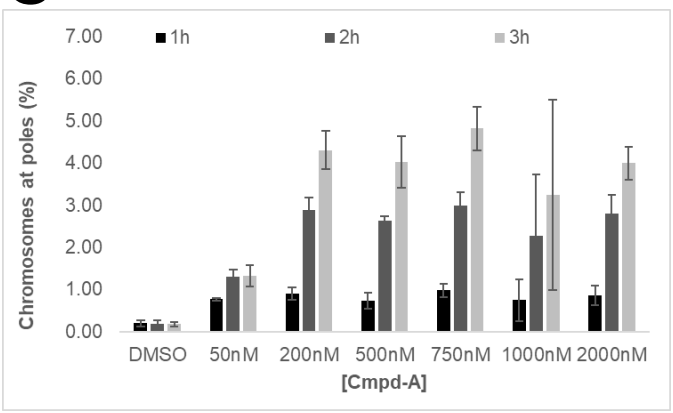
A



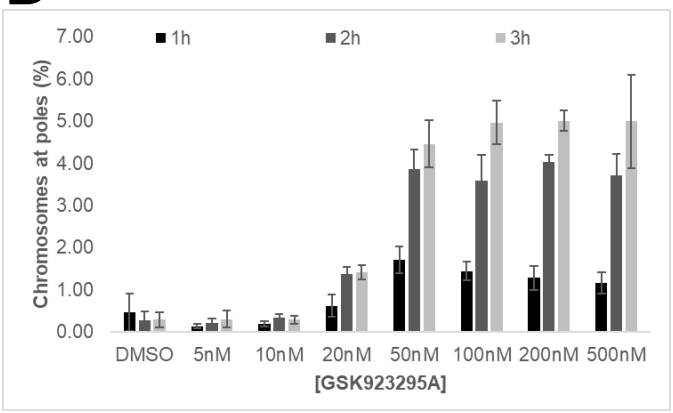
B



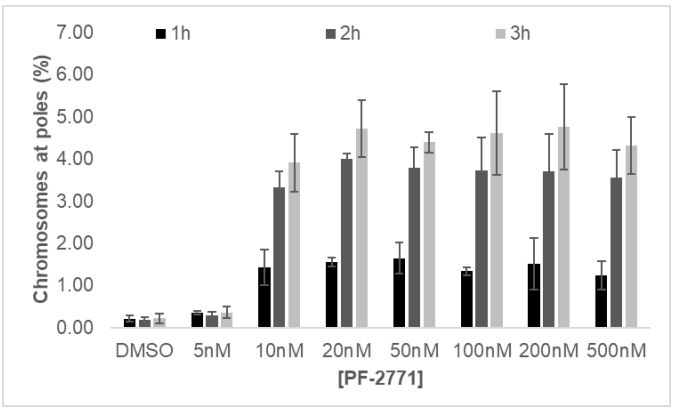
C



D



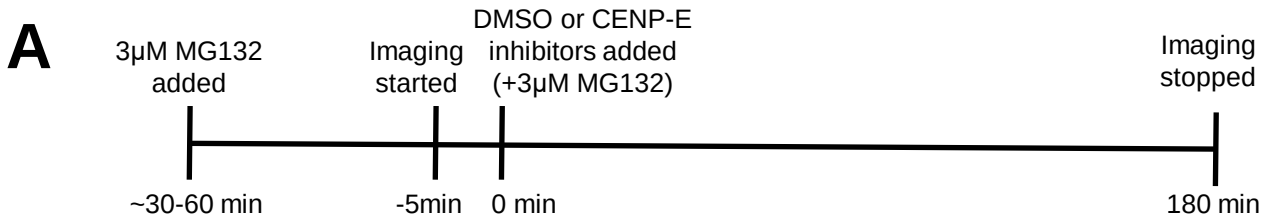
E

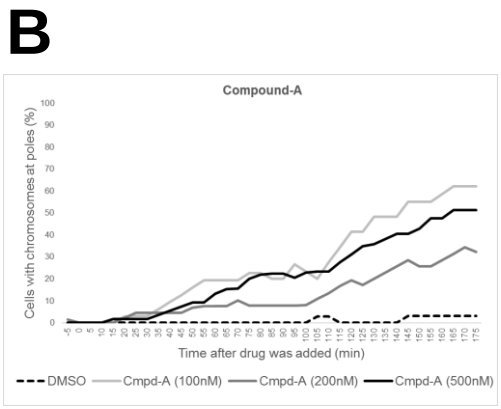


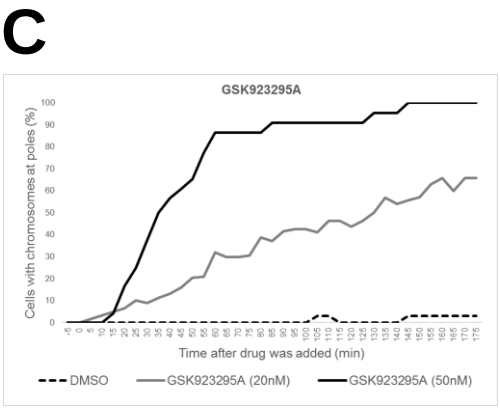
F

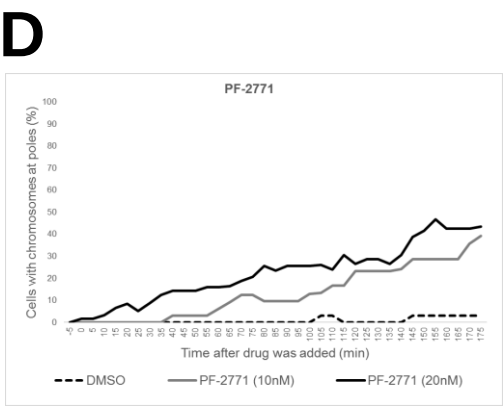
Compound/ Drug	Inhibition mechanism	Inhibitor binding site	Clinical Trials
Cmpd-A	Competitive towards ATP	ATP binding site on CENP-E	Not considered
GSK923295A	Allosteric (induces rigor-like state)	Between helices α2 and α3 on CENP-E motor domain (induces a MT-bound state)	Phase I concluded (2012)
PF-2771	Non-competitive towards ATP	Unknown (decreases CENP-E affinity for ATP)	Not considered

Figure 1 – Titration of CENP-E inhibitors in HeLa cells. Representative images of **(A)** DMSO and **(B)** GSK923295A-treated HeLa cells expressing H2B-GFP after a 3hr treatment. Images acquired using a 20X objective mounted on an IN Cell Analyzer 2000 microscope system. Insets show 5X magnifications of selected regions exemplifying different cellular phenotypes. Images collected were analysed using a custom-made CellProfiler pipeline and the percentage of cells displaying chromosomes at poles (over the total number of cells) is shown for cells treated with **(C)** Cmpd-A, **(D)** GSK923295A and **(E)** PF-2771. Error bars represent SD for quaduplicate experiments. **(F)** Table summarizing chemical properties of CENP-E inhibitors


A
3μM MG132 added
Imaging started
DMSO or CENP-E inhibitors added (+3μM MG132)
Imaging stopped
~30-60 min
-5min
0 min
180 min


B
Compound-A
Cells with chromosomes at poles (%)
Time after drug was added (min)
DMSO
Cmpd-A (100nM)
Cmpd-A (200nM)
Cmpd-A (500nM)


C
GSK923295A
Cells with chromosomes at poles (%)
Time after drug was added (min)
DMSO
GSK923295A (20nM)
GSK923295A (50nM)


D
PF-2771
Cells with chromosomes at poles (%)
Time after drug was added (min)
DMSO
PF-2771 (10nM)
PF-2771 (20nM)

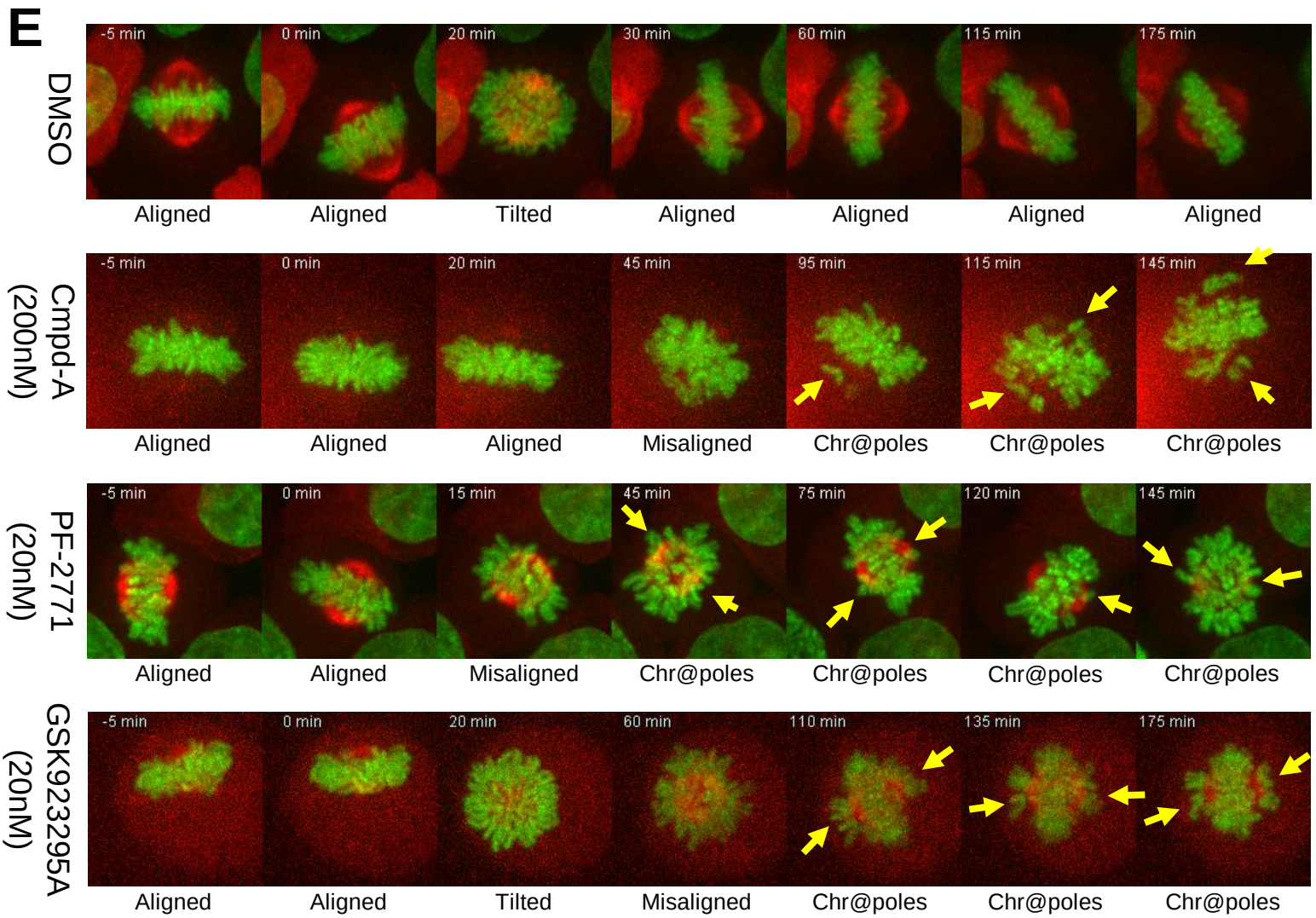

E
DMSO
-5 min
0 min
20 min
30 min
60 min
115 min
175 min
Aligned
Aligned
Tilted
Aligned
Aligned
Aligned
Aligned
Cmpd-A (200nM)
-5 min
0 min
20 min
45 min
95 min
115 min
145 min
Aligned
Aligned
Aligned
Misaligned
Chr@poles
Chr@poles
Chr@poles
PF-2771 (20nM)
-5 min
0 min
15 min
45 min
75 min
120 min
145 min
Aligned
Aligned
Misaligned
Chr@poles
Chr@poles
Chr@poles
Chr@poles
GSK923295A (20nM)
-5 min
0 min
20 min
60 min
110 min
135 min
175 min
Aligned
Aligned
Tilted
Misaligned
Chr@poles
Chr@poles
Chr@poles

Figure 2 – CENP-E is required for the maintenance of chromosome alignment. (A) Schematic representation of the experimental setup used to assay CENP-E function in the maintenance of chromosome alignment. HeLa cells were treated with MG132 (3μM) for ~30min. Cells were selected for imaging on the basis of chromosome alignment and imaging started 5 min before the addition of DMSO or CENP-E inhibitors. MG132 (3μM) was maintained in the media containing DMSO or CENP-E inhibitors. Cells were followed through live cell imaging for a further 180min. Quantifications of the percentage of HeLa cells that display chromosomes at poles after treatment with different concentrations of **(B)** Cmpd-A **(C)** GSK923295A or **(D)** PF-2771. **(E)** Selected time-frames of represenative cells treated with CENP-E inhibitors demonstrating the progressive misalignment of congressed chromosomes and the subsequent formation of chromosomes at the poles. Please note that while DMSO-treated cells remain mostly aligned throughout the experiment, most of the CENP-E-inhibited cells display misalgned chromosomes that often result in the formation of chromosomes at poles. Yellow arrows indicate chromosomes at poles.

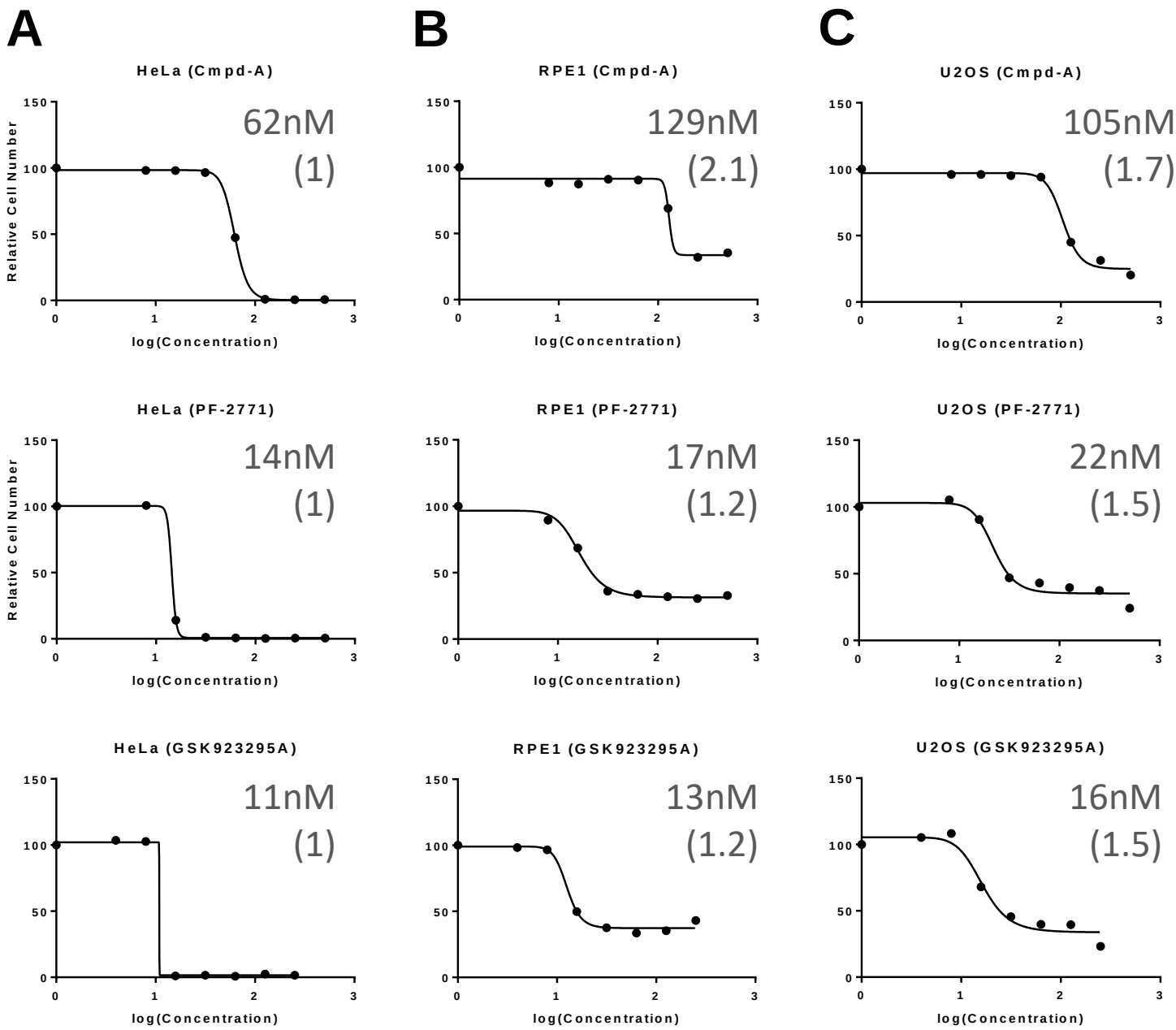
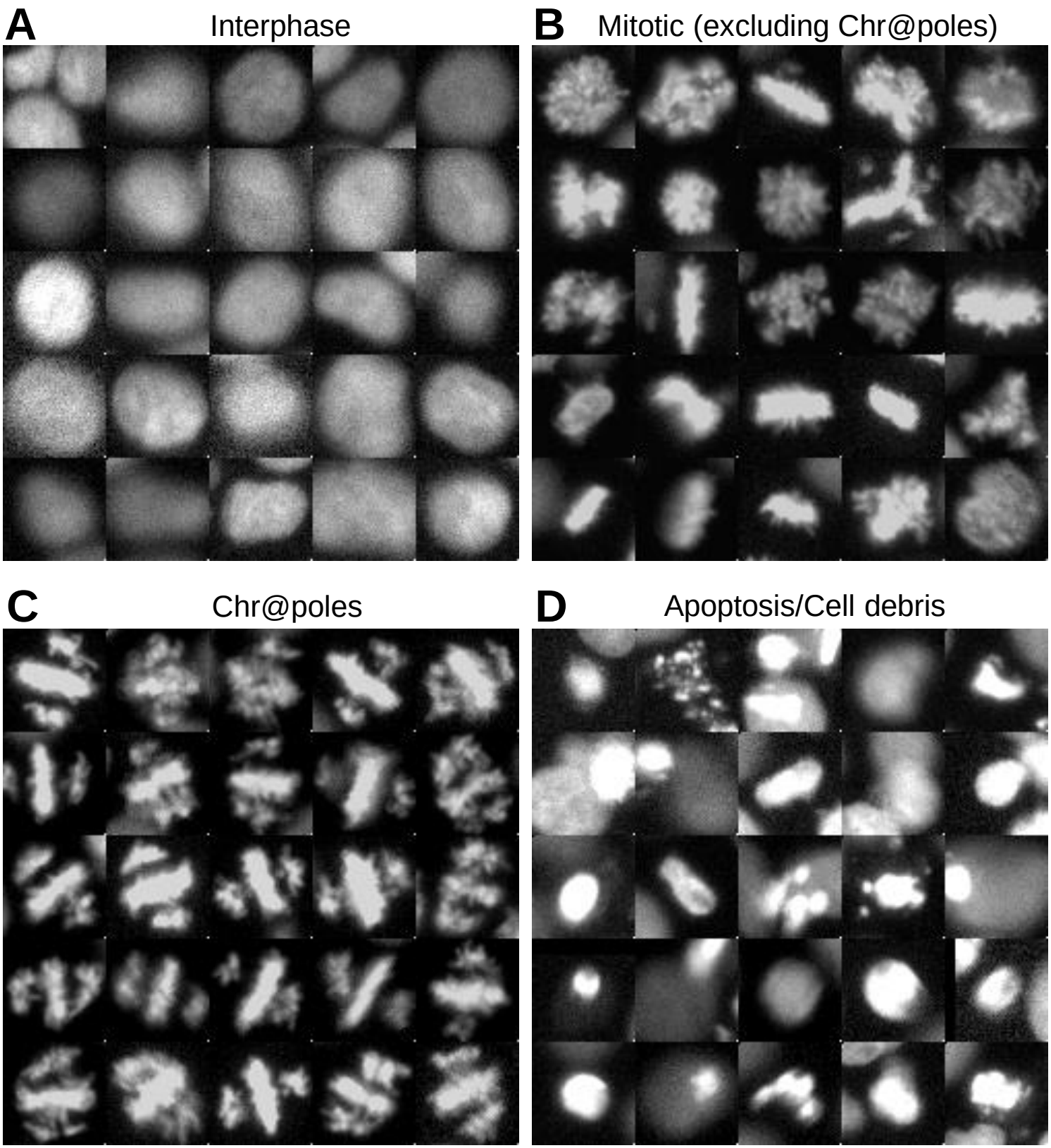
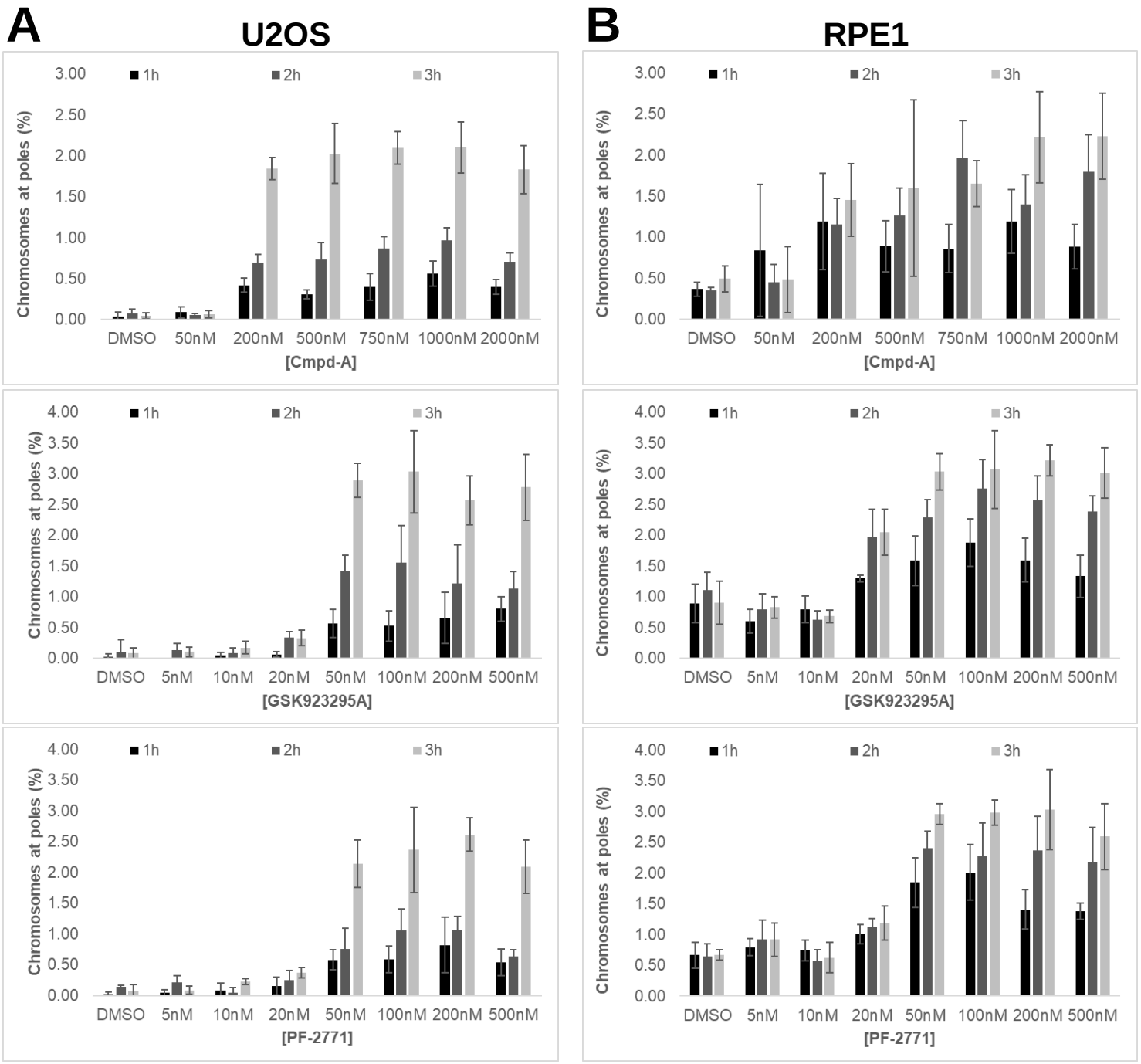


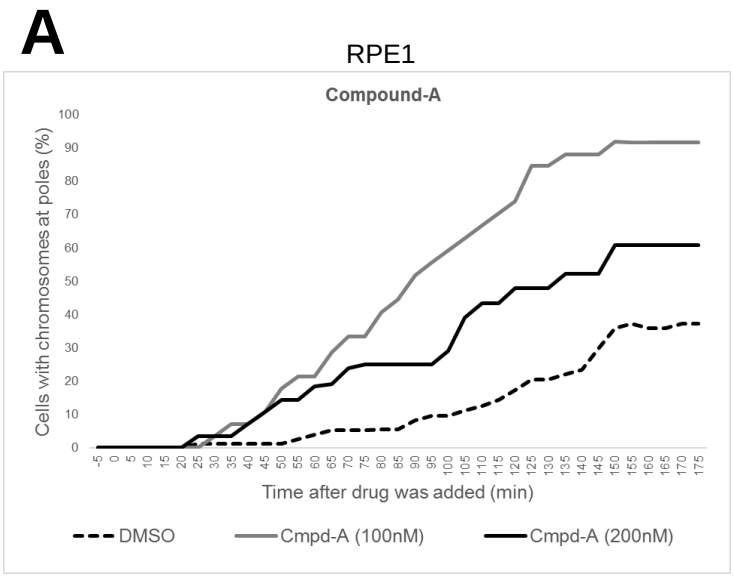
Figure 3 – Diploid cells are less sensitive to Cmpd-A-mediated cell death . (A) HeLa, **(B)** RPE1 and **(C)** U2OS cells were treated with varying concentrations of Cmpd-A (top row), GSK923295A (middle row) or PF-2771 (bottom row) and the relative cell number was calculated 5 days after addition of the drugs. Relative cell number was calculated using a custom-generated Resazurin Cell Viabiliy assay. Calculated (represented in nM) and relative (represented below as -fold of HeLa cells) IC50 values are shown in gray in the top right corner of each graph.

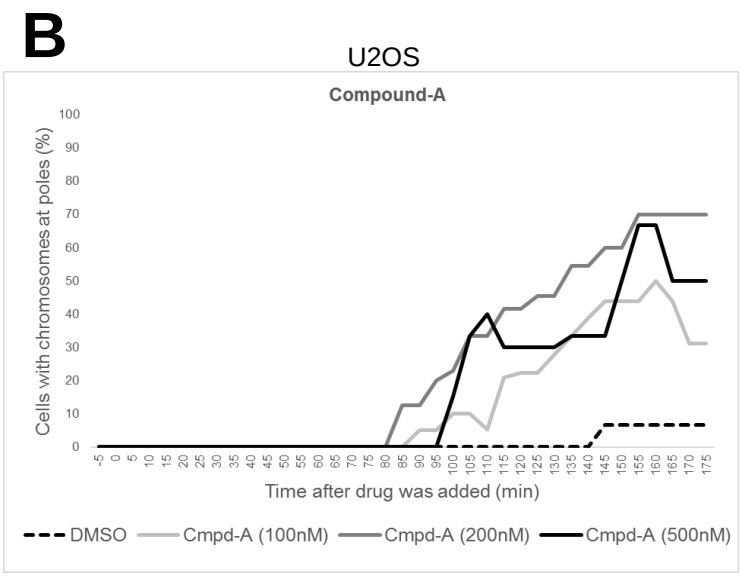


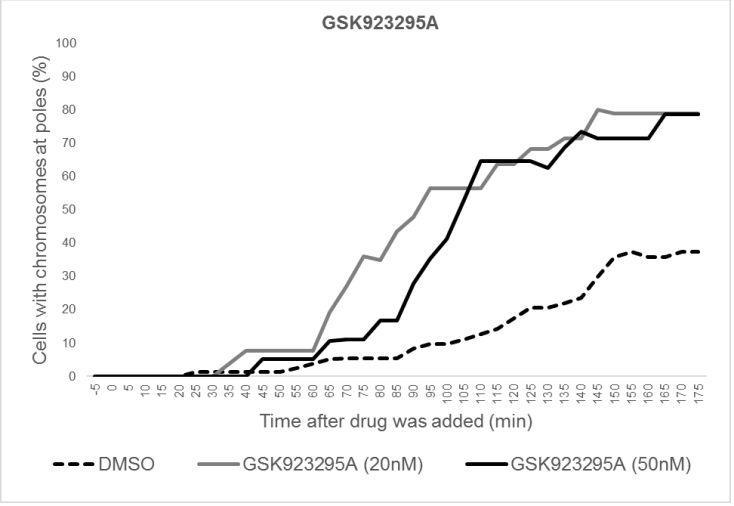
Supplementary Figure 1 – CellProfiler2.2.0 image training gallery used for quantifying cellular state. Panels show randomized images used to train CellProfiler Analyst software using the following cellular classes: **(A)** Interphase, **(B)** Mitotic (mitotic cells without chromosomes at poles), **(C)** Mitotic cells with chromosomes at poles or **(D)** Apoptotic/Cell debris.

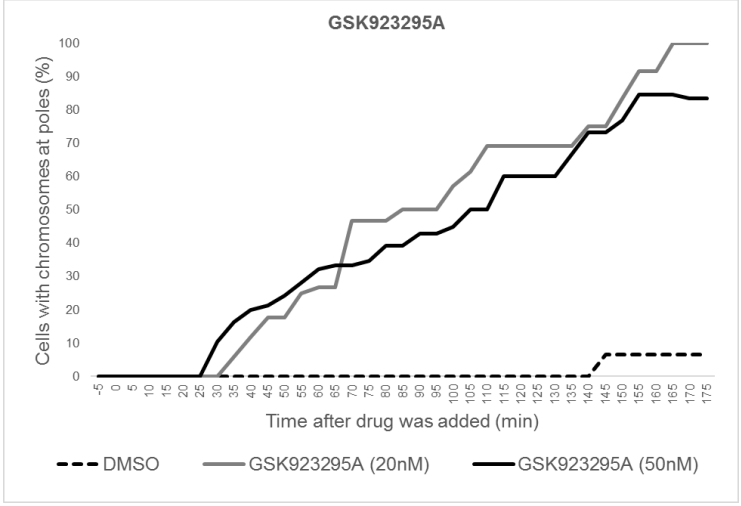


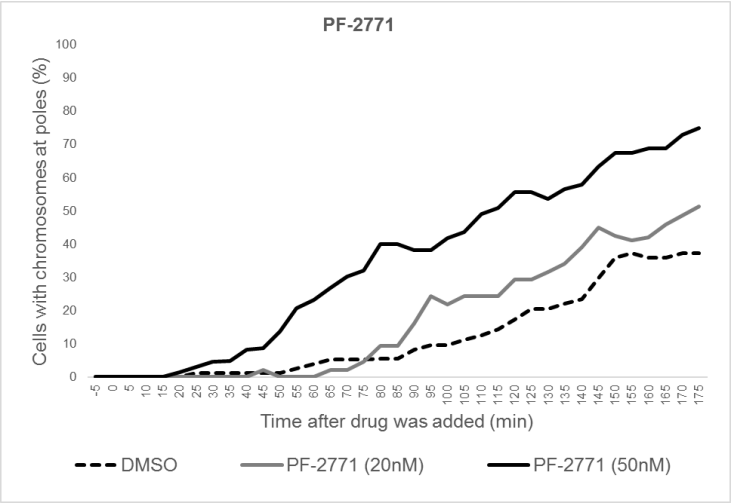
Supplementary Figure 2 – Titration of CENP-E inhibitors in U2OS and RPE1 cells. CellProfiler analysis of the percentage of cells displaying chromosomes at poles (over the total number of cells) is shown for cells treated with **(A)** U2OS and **(B)** RPE1 cells. Error bars represent SD for quaduplicate experiments.

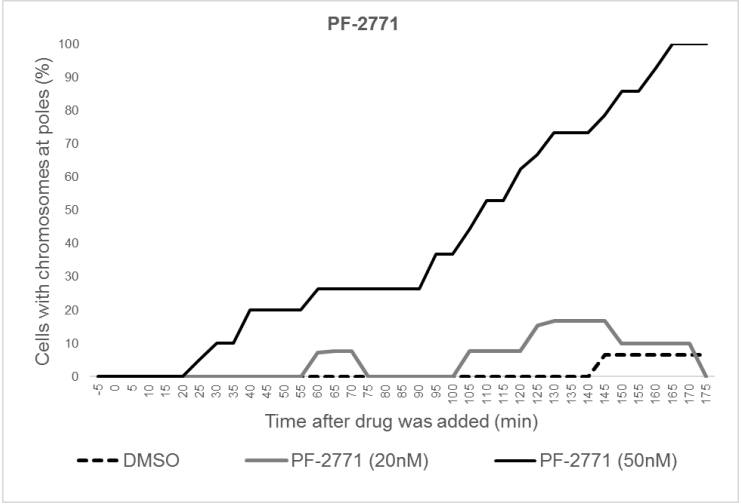




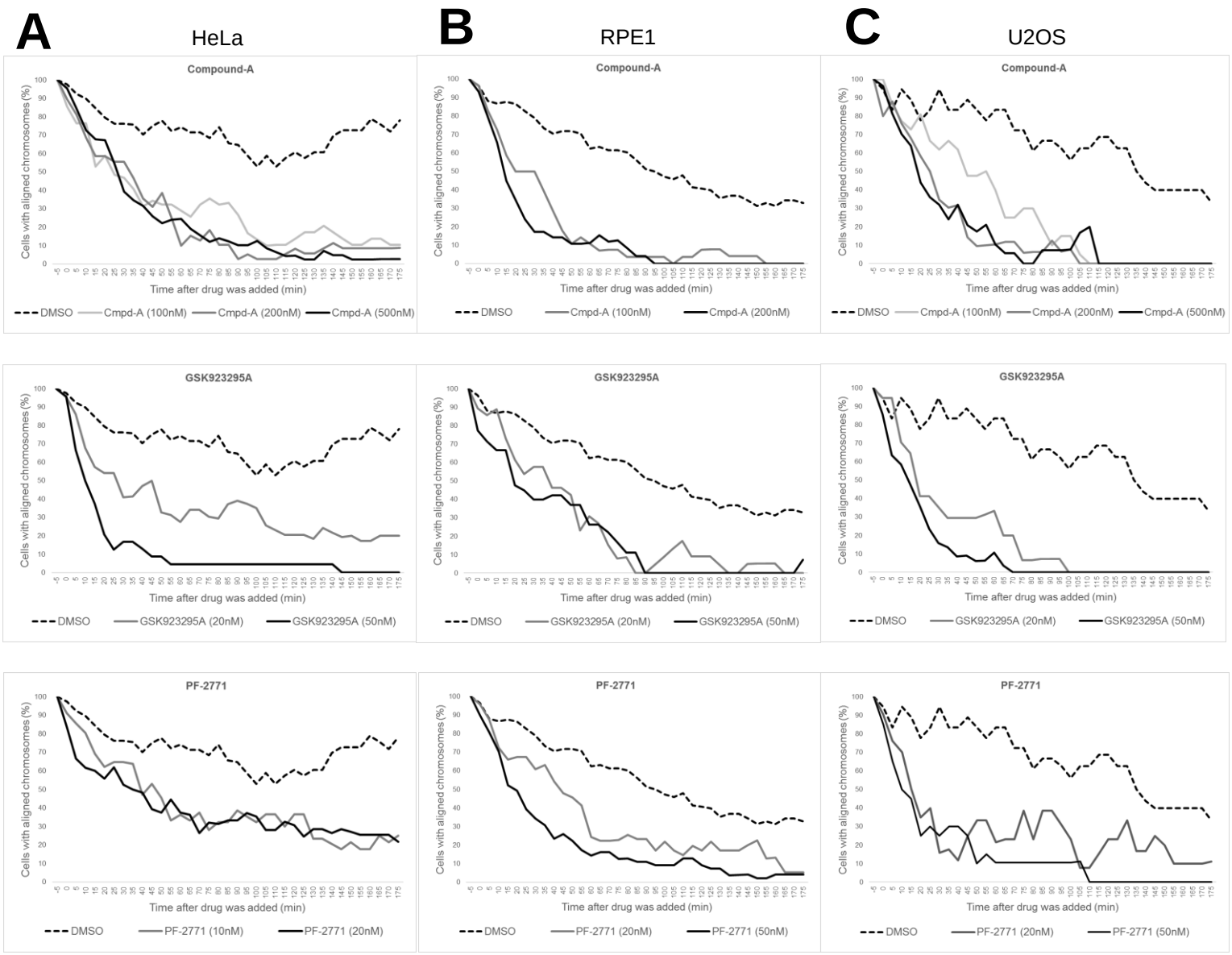




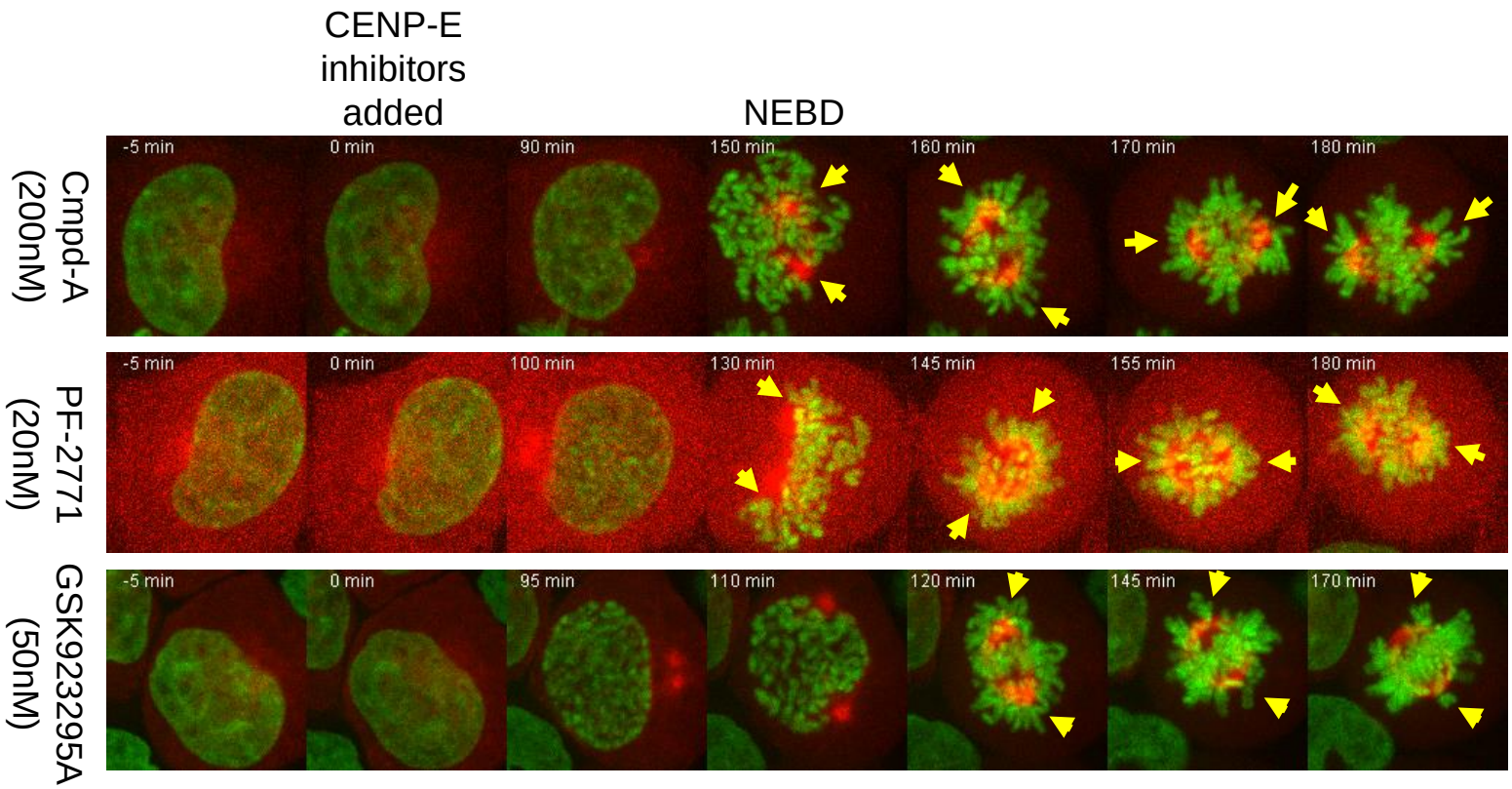




Supplementary Figure 3 – Inhibiting CENP-E function during metaphase causes chromosomes at poles in RPE1 and U2OS cells. Quantification of the percentage of cells with chromosomes at poles for **(A)** RPE1 or **(B)** U2OS cells treated with Cmpd-A (top row), GSK923295A (middle row) or PF2771 (bottom row). Results were obtained from the analysis of live cell imaging. Please note that cells undergoing any of the terminal events were removed from the analysis from that time point onwards.



Supplementary Figure 4 – Inhibiting CENP-E function during metaphase induces chromosome misalignment. Quantification of the percentage of cells that remain aligned during imaging for (A) HeLa, (B) RPE1 or (C) U2OS cells treated with Cmpd-A (top row), GSK923295A (middle row) or PF2771 (bottom row). Results were obtained from the analysis of live cell imaging. Please note that cells undergoing any of the terminal events were removed from the analysis from that time point onwards.



Supplementary Figure 5 – Addition of CENP-E inhibitors prior to NEBD induces chromosomes at poles. Selected time frames of representative HeLa cells treated with **(A)** Cmpd-A (200nM) **(B)** PF-2771 (20nM) or **(C)** GSK923295A (20nM) for at least 100min before NEBD. Yellow arrows indicate chromosomes at poles.

A

HeLa	Anaphase	Out of focus	Cohesion Fatigue	Mitotic slippage
DMSO	10.3	0.0	12.8	0.0
Cmpd-A (100nM)	16.7	0.0	3.3	0.0
Cmpd-A (200nM)	23.3	0.0	9.3	0.0
Cmpd-A (500nM)	38.7	0.0	12.9	0.0
GSK923295A (20nM)	49.2	0.0	1.6	0.0
GSK923295A (50nM)	8.7	0.0	0.0	4.3
PF-2771 (10nM)	36.6	0.0	7.3	2.4
PF-2771 (20nM)	21.6	0.0	7.8	0.0
PF-2771 (50nM)	Not imaged	Not imaged	Not imaged	Not imaged

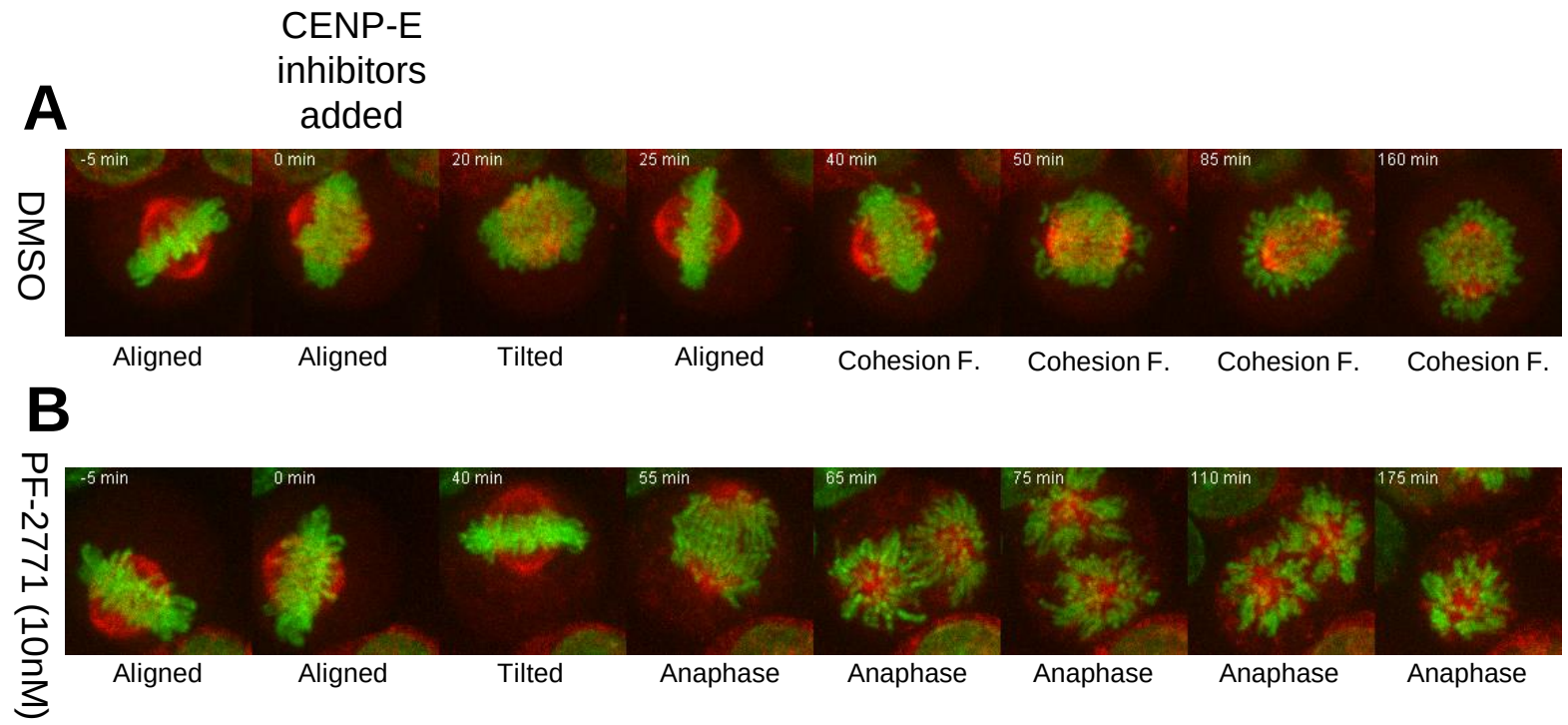
B

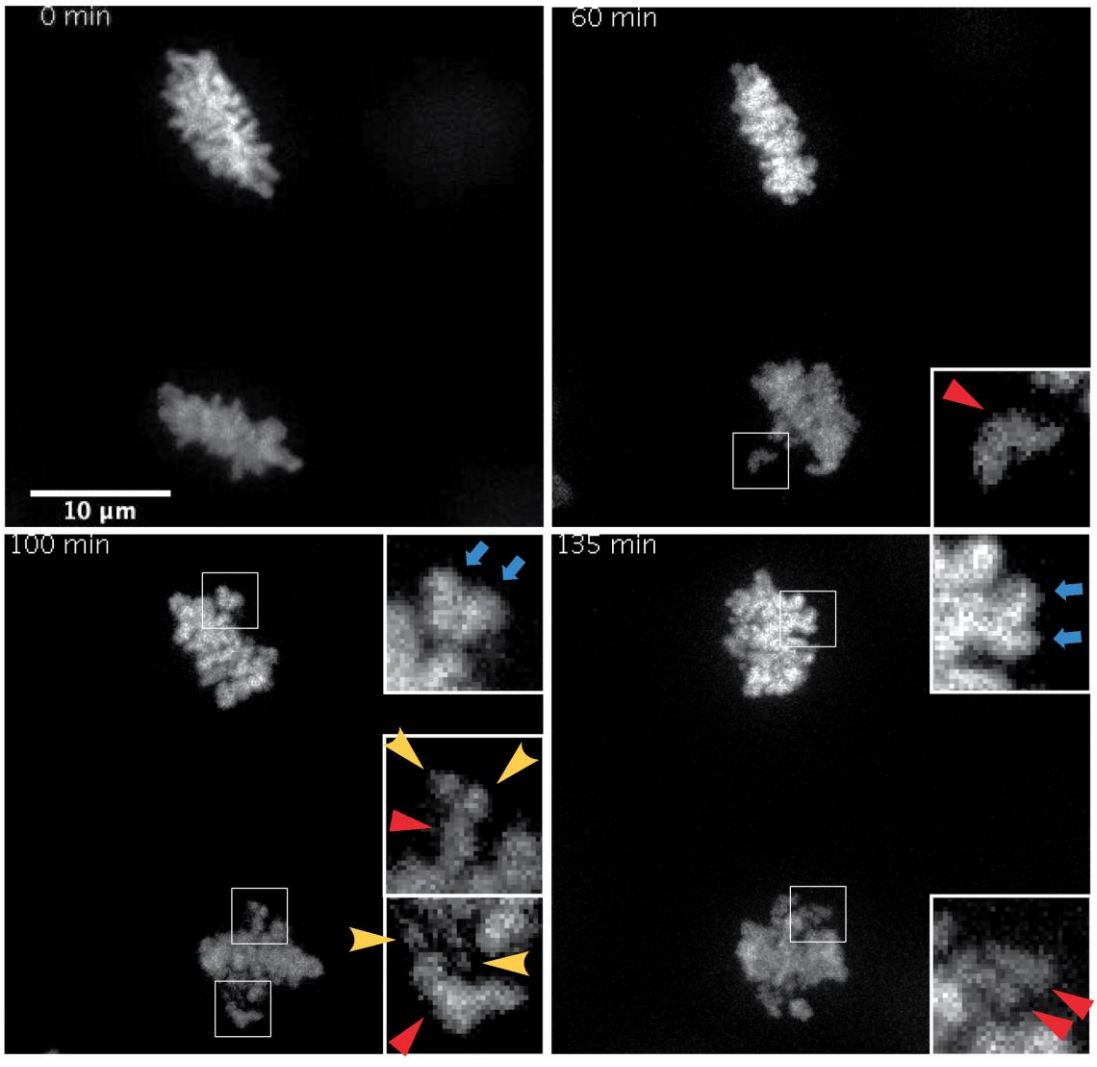
RPE1	Anaphase	Out of focus	Cohesion Fatigue	Mitotic slippage
DMSO	2.5	13.6	4.9	0.0
Cmpd-A (100nM)	0.0	10.7	3.6	0.0
Cmpd-A (200nM)	3.7	3.7	7.4	0.0
Cmpd-A (500nM)	Not imaged	Not imaged	Not imaged	Not imaged
GSK923295A (20nM)	8.0	28.0	0.0	0.0
GSK923295A (50nM)	11.8	29.4	0.0	0.0
PF-2771 (10nM)	Not imaged	Not imaged	Not imaged	Not imaged
PF-2771 (20nM)	4.3	17.4	0.0	0.0
PF-2771 (50nM)	32.4	11.3	8.5	0.0

C

U2OS	Anaphase	Out of focus	Cohesion Fatigue	Mitotic slippage
DMSO	0.0	0.0	11.8	0.0
Cmpd-A (100nM)	0.0	4.5	22.7	0.0
Cmpd-A (200nM)	0.0	0.0	75.0	0.0
Cmpd-A (500nM)	3.8	0.0	76.9	0.0
GSK923295A (20nM)	0.0	0.0	35.3	0.0
GSK923295A (50nM)	0.0	0.0	77.8	0.0
PF-2771 (10nM)	Not imaged	Not imaged	Not imaged	Not imaged
PF-2771 (20nM)	21.1	0.0	36.8	0.0
PF-2771 (50nM)	0.0	0.0	35.3	0.0

Supplementary Figure 6 – Terminal events that ocurred during time-lapse imaging. Tables indicate the percentage of cells that undergo any of the terminal events shown for **(A)** HeLa, **(B)** RPE1 and **(C)** U2OS cells. Please note that each terminal event happened only once in each cell and those cells were excluded from the analysis of chromosome alignment from that point onwards.

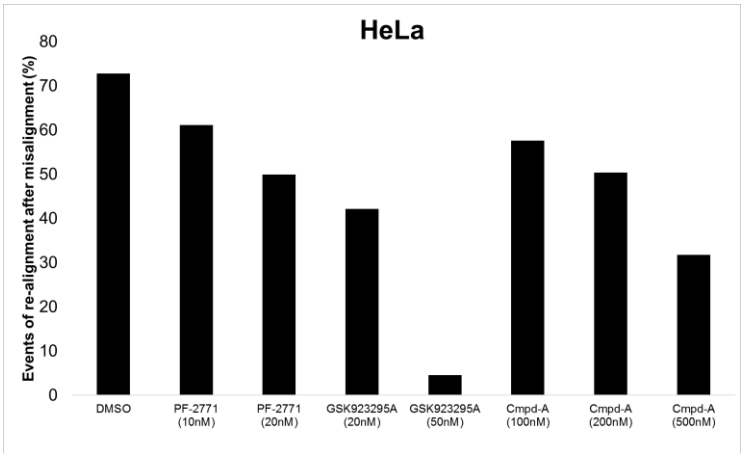


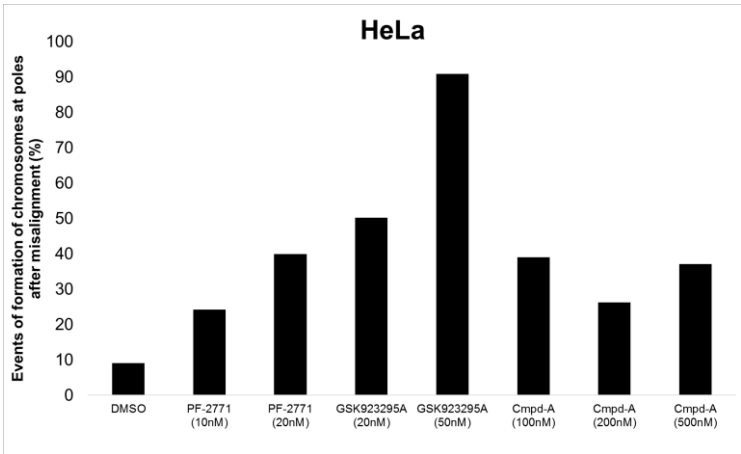


Supplementary Figure 7 – Treatment with CENP-E inhibitors induces mitotic exit through cohesion fatigue and anaphase. Selected time frames of representative HeLa cells treated with **(A)** DMSO or **(B)** PF-2771 (10nM) showing representative examples of cohesion fatigue and anaphase, respectively. **(C)** Selected time-frames of maximum intensity projections of U2OS cells treated with Cmpd-A (100nM).

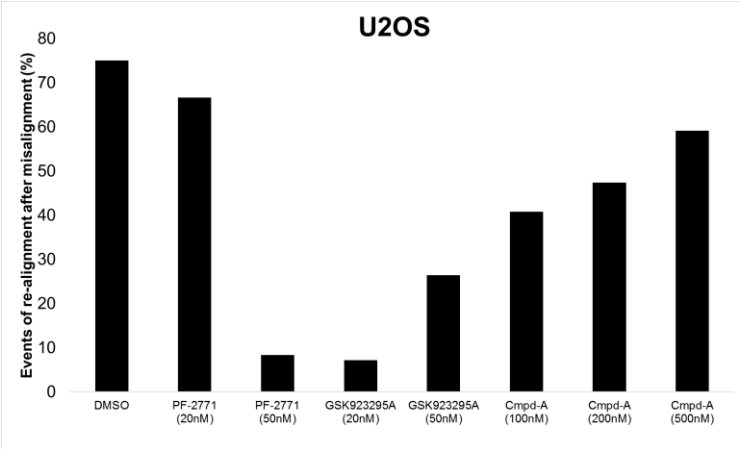
Insets represent 3X magnifications of selected regions. Red arrowheads indicate single chromatids; yellow arrowheads indicate chromosome fragments (consistent with cohesion fatigue) and blue arrows indicate paired sister chromatids organized as chromosomes.

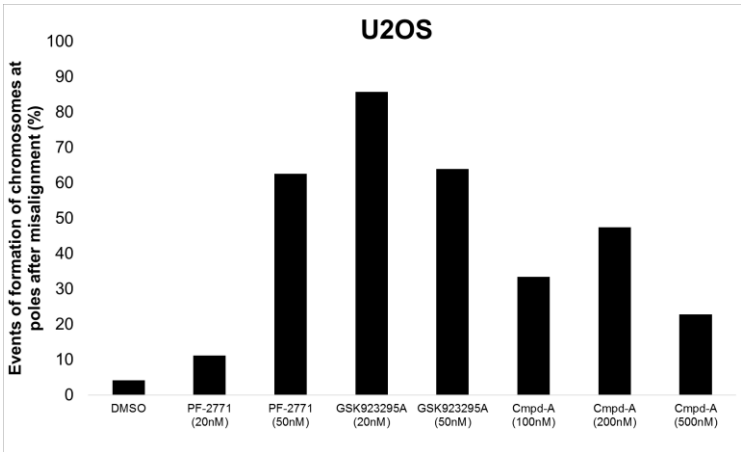
A



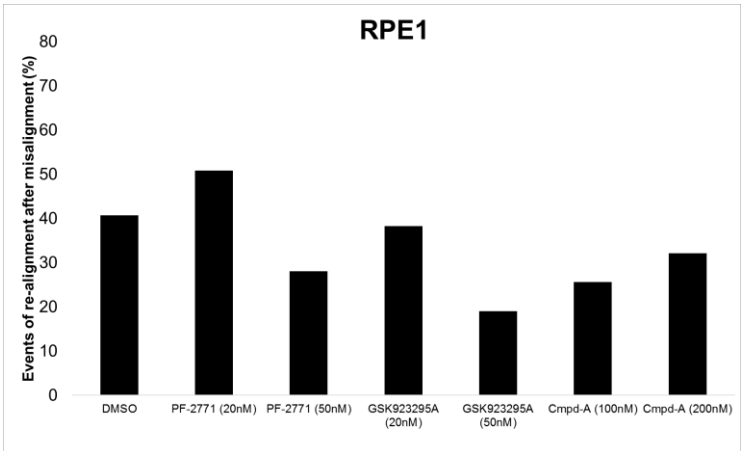


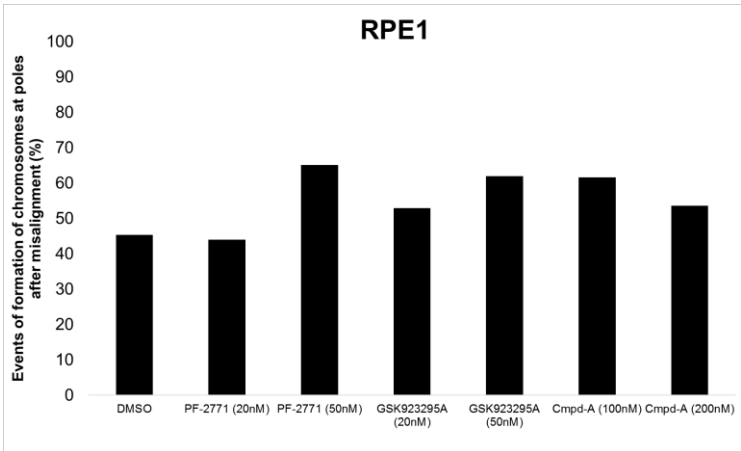
B





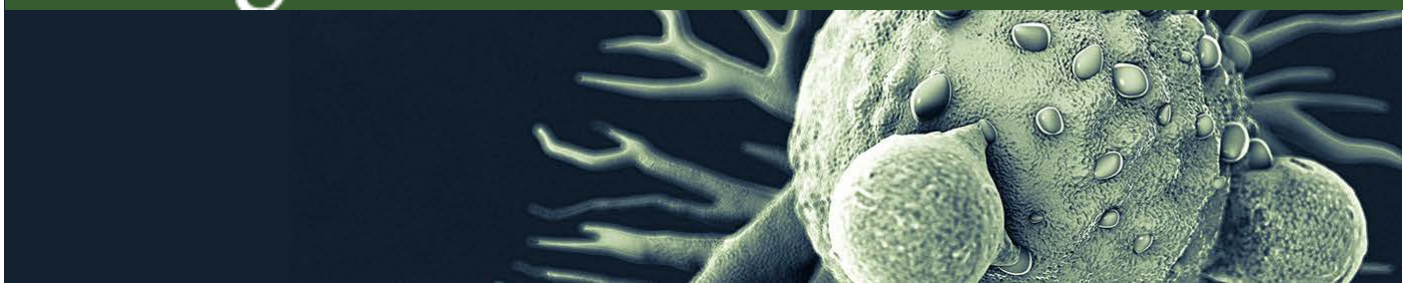
C





Supplementary Figure 8 – CENP-E is required for chromosome re-alignment. Percentage of alignment events after misalignment (left graph) and percentage of cells that generate chromosomes at poles after misalignment (right graph) for **(A)** HeLa, **(B)** U2OS or **(C)** RPE1 cells.

Oncogene



ABOUT THE JOURNAL

Aims and Scope

Oncogene aims to make substantial advances in our knowledge of the processes that contribute to cancer by publishing outstanding research. We propagate work that challenges standard conjecture and builds on previous studies, in particular those that lead to establishing new paradigms in the etio-pathogensis, diagnosis, treatment, or prevention of cancers, and in processes that drive metastatic spread, and provide important insights into cancer biology beyond what has been revealed thus far. Areas covered by *Oncogene* include but are not limited to, the cellular and molecular biology of cancer including resistance to cancer therapies, and development of better approaches to improving survival. Across cancer biology, we have a broad remit encompassing the life sciences and biomedicine, from the most fundamental and theoretical work, through to translational, applied, and clinical research. Our Editors are among the most respected and accomplished individuals in their fields – from genetics and structural biology, to function and mechanism.

Journal Details

Editorial office:
Springer Nature, One New York Plaza, Suite 4500
New York, NY 10004-1562
oncogene@us.nature.com

Impact factor: 7.519 (*2016 Journal Citation Reports,* Clarivate Analytics, 2017)

Frequency: 50 issues a year

Abstracted in:

EBSCO Discovery Service
Google Scholar
OCLC
Summon by ProQuest
BIOSIS
Current Contents/Life Sciences
Science Citation Index
Science Citation Index Expanded (SciSearch)
SCOPUS
EBSCO Academic Search
EBSCO Advanced Placement Source
EBSCO Biomedical Reference Collection
EBSCO Science & Technology Collection
EBSCO STM Source
EBSCO TOC Premier
INIS Atominde

ARTICLE TYPE SPECIFICATIONS

ARTICLE DESCRIPTION	SPECIFICATION
Article An Article is a substantial, in-depth, novel research study of interest to the readership of the journal. The structure an Article should follow is detailed below.	Untructured abstract, max 250 words. Main body of text (excluding abstract, tables/figures, and references) not to exceed 4,500 words; Max 8 tables or figures; Max 80 references
Review Article A Review Article is an authoritative, balanced survey of recent developments in a research field. Review Articles should incorporate a) a review of previously published literature from the past 5-10 years, describing the pros and cons of these studies, b) the authors opinion on how to approach the issue/situation being discussed, c) the authors thoughts on what is necessary to move the field forward in the future. Review Articles are regularly commissioned, however pre-submission enquiries are also welcome. Please contact oncogene@us.nature.com	Unstructured abstract, max 300 words. Main body of text (excluding abstract, tables/figures, and references) not to exceed 6,000 words; Max 8 tables or figures; Max 100 references

Brief Communication A brief communication is a concise, independent report representing a significant and timely contribution to cancer biology. A short communication is not intended to publish preliminary results. The results must be of exceptional interest and relevant to be considered for publication.	Unstructured abstract, max 200 words. Main body of text (excluding abstract, tables/figures, and references) not to exceed 2,500 words; Max 4 tables or figures; Max 40 references
Correspondence Correspondence provides readers with a forum for comment on papers published in a previous issue of the journal or to address new issues relevant to the research community.	No abstract required Main body of text (excluding abstract, tables/figures, and references) not to exceed 1,000 words; Max 2 tables or figures; Max 20 references

Special Issues

Special issues are comprised of a group of high quality, peer-reviewed manuscripts about a single specific theme / topic. Although the individual manuscripts are stand alone, they collectively make an important point by offering a comprehensive view, or by providing a diverse perspective. The number of manuscripts in a special issue is determined on case by case basis.

Special Issues are commissioned only by invitation or upon consultation with *Oncogene* editorial staff. Please contact Editors-in-Chief, Justin Stebbing (justinstebbing@gmail.com) or George Miller (George.Miller@nyumc.org) for preliminary inquiries about special issues. Usually, a person willing to be the Guest Editor of special issue should initiate this process. This Guest Editor will act as the point of contact between *Oncogene* and the individual authors submitting manuscripts.

PREPARATION OF ARTICLES

House Style: Authors should adhere to the following formatting guidelines

- Text should be double spaced with a wide margin.
- All pages and lines are to be numbered.
- Do not make rules thinner than 1pt (0.36mm).
- Use a coarse hatching pattern rather than shading for tints in graphs.
- Colour should be distinct when being used as an identifying tool.
- Spaces, not commas should be used to separate thousands.
- At first mention of a manufacturer, the town (and state if USA) and country should be provided.
- Statistical methods: For normally distributed data, mean (SD) is the preferred summary statistic. Relative risks should be expressed as odds ratios with 95% confidence interval. To compare two methods for measuring a variable the method of Bland & Altman (1986, Lancet 1, 307–310) should be used; for this, calculation of P only is not appropriate.
- Units**:** Use metric units (SI units) as fully as possible. Preferably give measurements of energy in kiloJoules or MegaJoules with kilocalories in parentheses (1 kcal = 4.186kJ). Use % throughout.
- Abbreviations: On first using an abbreviation place it in parentheses after the full item. Very common abbreviations such as FFA, RNA, need not be defined. Note these abbreviations: gram g; litre l; milligram mg; kilogram kg; kilojoule kJ; megajoule MJ; weight wt; seconds s; minutes min; hours h. Do not add 's' for plural units. Terms used less than four times should not be abbreviated.

Please note that original articles must contain the following components. Please see below for further details.

- Cover letter (including a Conflict of interest statement)
- Title page (excluding acknowledgements)
- Abstract and keywords
- Introduction
- Results
- Discussion
- Materials (or Subjects) and methods
- Acknowledgements
- Conflict of Interest
- References
- Figure legends
- Tables
- Figures

Cover Letter

Authors should provide a cover letter that includes the affiliation and contact information for the corresponding author. Authors should briefly discuss the importance of the work and explain why it is considered appropriate for the diverse readership of the journal. The cover letter should confirm the material is original research, has not been previously published and has not been submitted for publication elsewhere while under consideration. If the manuscript has been previously considered for publication in another journal, please include the previous reviewer comments, to help expedite the decision by the Editorial team. Please also include a Conflict of Interest statement, see Editorial Policies for more details.

The title page should contain:

- Title of the paper - brief, informative, of 150 characters or less and should not make a statement or conclusion
- Running title – should convey the essential message of the paper in no more than 50 characters. Should not contain any abbreviations
- Full names of all the authors and their affiliations, together with the name, full postal address, telephone number and e-mail address of the corresponding author. If authors regard it as essential to indicate that two or more co-authors are equal in status, they may be identified by an asterisk symbol with the caption 'These authors contributed equally to this work' immediately under the address list.

Group Authorship/Collaborations - Please note that if in the list of authors you wish to include additional authors/collaborators/ groups/consortiums that aren't part of the core list of authors as 'on behalf of', 'for the' or 'representing the' you need to ensure you list the authors correctly within the paper to ensure these are there deposited correctly in PubMed.

- Groups where there is an 'on behalf of', or 'representing the', or 'for the' will appear in the HTML/PDF as follows: Author A, Author B, Author C and Author D on behalf of…The list of individual members should then appear in the Acknowledgements section and not under Notes or Appendix
- A Group name who is an author in its own right should have the list of authors as usual and then all the individual authors of the group listed in their own section at the end of the article, NOT in Acknowledgement/Appendix or Notes

- Competing Interests statement (see Editorial Policy section). Authors should disclose the sources of any support for the work received in the form of grants and/or equipment and drugs.

Abstract
Articles must be prepared with an unstructured abstract designed to summarise the essential features of the paper in a logical and concise sequence.

Introduction
The Introduction should assume that the reader is knowledgeable in the field and should therefore be as brief as possible but can include a short historical review where desirable.

Results
The Results section should briefly present the experimental data in text, tables or figures. Tables and figures should not be described extensively in the text.

Discussion
The Discussion should focus on the interpretation and the significance of the findings with concise objective comments that describe their relation to other work in the area. It should not repeat information in the results. The final paragraph should highlight the main conclusion(s), and provide some indication of the direction future research should take.

Materials and Methods
Materials and methods must contain sufficient details, including references, so that all experimental procedures can be reproduced. Methods that have been published in detail elsewhere should not be described in detail; a reference to the original paper should be used instead.
Authors should provide the name of the manufacturer and their location for any specifically named medical equipment and instruments, and all drugs should be identified by their pharmaceutical names, and by their trade name if relevant.
Authors must identify the source and sequences of siRNA or shRNA used in experiments. *Oncogene* does not publish papers that employ only a single siRNA or shRNA.

Acknowledgements
These should be brief, and should include sources of support including sponsorship (e.g. university, charity, commercial organisation) and sources of materials (e.g. novel drugs) not available commercially.

Competing Interests
Authors must declare whether or not there are any competing financial interests in relation to the work described. This information must be included at this stage and will be published as part of the paper, but should also be noted in the cover letter and on the title page. Please see the Competing Interests definition in the Editorial Policy section for detailed information.

References
Only papers directly related to the article should be cited. Exhaustive lists should be avoided. References should follow the Vancouver format. In the text they should appear as numbers starting at one and at the end of the paper they should be listed (double-spaced) in numerical order corresponding to the order of citation in the text. Where a reference is to appear next to a number in the text, for example following an equation, chemical formula or biological acronym, citations should be written as (ref. X) and not as superscript.
Example "detectable levels of endogenous Bcl-2 (ref. 3), as confirmed by western blot"

All authors should be listed for papers with up to six authors; for papers with more than six authors, the first six only should be listed, followed by *et al.*
Abbreviations for titles of medical periodicals should conform to those used in the latest edition of Index Medicus. The first and last page numbers for each reference should be provided. Abstracts and letters must be identified as such. Papers in press may be included in the list of references.
Personal communications can be allocated a number and included in the list of references in the usual way or simply referred to in the text; the authors may choose which method to use. In either case authors must obtain permission from the individual concerned to quote his/her unpublished work.

Examples:
Journal article:
Belkaid Y, Rouse BT. Natural regulatory T cells in infectious disease. *Nat Immunol* 2005; **6**: 353–360.

Journal article, e-pub ahead of print:
Bonin M, Pursche S, Bergeman T, Leopold T, Illmer T, Ehninger G *et al*. F-ara-A pharmacokinetics during reduced-intensity conditioning therapy with fludarabine and busulfan. *Bone Marrow Transplant* 2007; e-pub ahead of print 8 January 2007; doi:10.1038/sj.bmt.1705565

Journal article, in press:
Gallardo RL, Juneja HS, Gardner FH. Normal human marrow stromal cells induce clonal growth of human malignant T-lymphoblasts. *Int J Cell Cloning* (in press).

Complete book:
Atkinson K, Champlin R, Ritz J, Fibbe W, Ljungman P, Brenner MK (eds). *Clinical Bone Marrow* and *Blood Stem Cell Transplantation*, 3rd edn. Cambridge University Press: Cambridge, UK, 2004.

Chapter in book:
Coccia PF. Hematopoietic cell transplantation for osteopetrosis. In: Blume KG, Forman SJ, Appelbaum FR (eds). *Thomas' Hematopoietic Cell Transplantation*, 3rd edn. Blackwell Publishing Ltd: Malden, MA, USA, 2004, pp 1443–1454.

Abstract:
Syrjala KL, Abrams JR, Storer B, Heiman JR. Prospective risk factors for five-year sexuality late effects in men and women after haematopoietic cell transplantation. *Bone Marrow Transplant* 2006; **37**(Suppl 1): S4 (abstract 107).

Correspondence:
Caocci G, Pisu S. Overcoming scientific barriers and human prudence [letter]. *Bone Marrow Transplant* 2006; **38**: 829–830.

Figure Legends
These should be brief, specific and appear on a separate manuscript page after the References section.

Tables
Tables should only be used to present essential data; they should not duplicate what is written in the text. **It is imperative that any tables used are editable**, ideally presented in Excel. Each must be uploaded as a separate workbook with a title or caption and be clearly labelled, sequentially. Please make sure each table is cited within the text and in the correct order, e.g. (Table 3). Please save the files with extensions .xls / .xlsx / .ods / or .doc or .docx. Please ensure that you provide a 'flat' file, with single values in each cell with no macros or links to other workbooks or worksheets and no calculations or functions.

Figures
Figures and images should be labelled sequentially and cited in the text. Figures should not be embedded within the text but rather uploaded as separate files. The use of three-dimensional histograms is strongly discouraged unless the addition of the third dimension is important for conveying the results. Composite figures containing more than three individual figures will count as two figures. All parts of a figure should be grouped together. Where possible large figures and tables should be included as supplementary material.

Detailed guidelines for submitting artwork can be found by downloading our Artwork Guidelines. Using the guidelines, please submit production quality artwork with your initial online submission. If you have followed the guidelines, we will not require the artwork to be resubmitted following the peer-review process, if your paper is accepted for publication

Graphs, Histograms and Statistics
- Plotting individual data points is preferred to just showing means, especially where N<10
- If error bars are shown, they must be described in the figure legend
- Axes on graphs should extend to zero, except for log axes
- Statistical analyses (including error bars and p values) should only be shown for independently repeated experiments, and must not be shown for replicates of a single experiment
- The number of times an experiment was repeated (N) must be stated in the legend

Supplementary Information
Supplementary information is peer-reviewed material directly relevant to the conclusion of an article that cannot be included in the printed version owing to space or format constraints. The article must be complete and self-explanatory without the Supplementary Information, which is posted on the journal's website and linked to the article. Supplementary Information may consist of data files, graphics, movies or extensive tables. Please see our Artwork Guidelines for information on accepted file types.

Authors should submit supplementary information files in the FINAL format as they are not edited, typeset or changed, and will appear online exactly as submitted. When submitting Supplementary Information, authors are required to:
- Include a text summary (no more than 50 words) to describe the contents of each file.
- Identify the types of files (file formats) submitted.
- Include the text "Supplementary information is available at (journal name)'s website" at the end of the article and before the references.

Video summaries
Oncogene allows authors to include video presentations as part of their submission in order to support and enhance their scientific research. Authors should include these videos as 'Supplementary Material' uploaded upon submission and can refer to these within the body of the text. This can be done in the same way you would upload any other supplementary information and the file should be clearly labelled 'Video Presentation'. Please take note of the technical requirements listed below. Videos supplied will be published online in the electronic version of your article, therefore please note that since they cannot be included in the print version of the journal, that you include text at the end of the article stating that 'Supplementary information is available on *Oncogene's* website'.

Tips for presentation
1. The video should introduce the topic of the article, highlight the main results and conclusions, discuss the current status and potential future developments in the field
2. Videos should be uploaded as Supplementary Material when submitting
3. Please include a sentence or two to describe the file. This will accompany your video on the website
4. Write your script and practise first – explain any obscure terminology
5. Film in a quiet room against a plain (white if possible) background and ensure there is nothing confidential in view
6. Avoid using background music
7. Include figures, slides, video clips of the experiment, etc. to help explain your methods and results. Please try to include a mixture of you talking to the camera and slides – it is nice for viewers to see your face at times
8. Keep figures simple; don't show raw data and ensure any text is legible. Do not include lots of small text or data that won't be legible in a small video player that's the size of a smartphone screen.

9. Please do not use images, music, or insignia in your video for which you do not own the copyright or have documented permission from the copyright holder.

Technical requirements
Videos should be no more than 8 minutes long, maximum 30MB in size so that they can be downloaded quickly - the combined total size of all supplementary files must not exceed 150 MB. Files should be submitted as .avi, .mov, .mp3, .mp4, .wav or .wmf. Videos need to be in widescreen (landscape), ideally 16x9 but 4:3 is also acceptable with a resolution of at least 640 x 360 pixels.
Any videos that are not in the correct format will not be published. Files will be viewed by the editorial office for quality; however the onus for creating, uploading and editing the video falls on the author.

Subject Ontology
Upon submission authors will be asked to select a series of subject terms relevant to the topic of their manuscript from our subject ontology. Providing these terms will ensure your article is more discoverable and will appear on appropriate subject specific pages on nature.com, in addition to the journal's own pages. Your article should be indexed with at least one, and up to four unique subject terms that describe the key subjects and concepts in your manuscript. Click here for help with this.

Language Editing
Oncogene is read by scientists from diverse backgrounds and many are not native English speakers. In addition, the readership of *Oncogene* is multidisciplinary; therefore authors need to ensure their findings are clearly communicated. Language and concepts that are well known in one subfield may not be well known in another. Thus, technical jargon should be avoided as far as possible and clearly explained where its use is unavoidable. Abbreviations, particularly those that are not standard, should also be kept to a minimum. The background, rationale and main conclusions of the study should be clearly explained and understandable by all working in the field. Titles and abstracts in particular should be written in language that will be readily understood by all readers.

Authors who are not native speakers of English sometimes receive negative comments from referees or editors about the language and grammar usage in their manuscripts, which can contribute to a paper being rejected. To reduce the possibility of such problems, we strongly encourage such authors to take at least one of the following steps.

- Have your manuscript reviewed for clarity by a colleague whose native language is English.
- Visiting the English language tutorial which covers the common mistakes when writing in English.
- Using a professional language editing service where editors will improve the English to ensure that your meaning is clear and identify problems that require your review. Two such services are provided by our affiliates Nature Research Editing Service and American Journal Experts.

Please note that the use of a language editing service is at the author's own expense and does not guarantee that the article will be selected for peer review or accepted

HOW TO SUBMIT

Pre-submission Enquiries
Oncogene implements a first round review of papers by our team of Deputy Editors to determine article suitability prior to full peer review. Please submit your full paper online for evaluation.

Online Submission
We only accept manuscript submission via our online manuscript submission system. Before submitting a manuscript, authors are encouraged to consult both our Editorial Policies and the Submission Instructions for our online manuscript submission system. If you have not already done so, please register for an account with our online manuscript system. You will be able to monitor the status of your manuscript online throughout the editorial process.

Summary of the editorial process

- The author submits a manuscript and it receives a tracking number.
- The editorial office perform an initial quality check on the manuscript to ensure that the paper is formatted correctly.
- A Deputy Editor is assigned to the manuscript and decides whether to send the manuscript out to review. If the decision is not to send the manuscript for review, the Editor-in-Chief contacts the author with the decision.
- If the Deputy Editordecides the paper is within the Journal's remit, the paper will be assigned to an Associate Editor.
- The Associate Editor selects and assigns peer reviewers. This can take some time dependent on the responsiveness and availability of the reviewers selected.
- Reviewers are given 14 days from acceptance to submit their reports. Once the required reports are submitted the Associate Editor will make a decision recommendation to the Editor-in-Chief based on the comments received.
- The Editor-in-Chief makes the final decision.

Authors are able to monitor the status of their paper throughout the peer review process

Peer review
To expedite the review process, only papers that seem most likely to meet editorial criteria are sent for external review. Papers judged by the editors to be of insufficient general interest or otherwise inappropriate are rejected promptly without external review.
Manuscripts sent out for peer review are evaluated by at least one independent reviewer (often two or more). Authors are welcome to suggest independent reviewers to evaluate their manuscript. All recommendations are considered, but it is at the Editor's discretion their choice of reviewers. By policy, referees

are not identified to the authors, except at the request of the referee.
Once a sufficient number of reviews are received, the editors then make a decision based on the reviewers' evaluations

- *Accept* - The manuscript is appropriate to be accepted as it stands.
- *Minor or major revision* - In cases where the editor determines that the authors should be able to address the referees' concerns in six months or less the editor may request a revised manuscript that addresses these concerns. The revised version is normally sent back to some or all of the original referees for re-review. The decision letter will specify a deadline for receipt of the revised manuscript and link via which the author should upload to the online submission system.
  When submitting a revision authors are asked to upload (1) A rebuttal letter, indicating point-by-point how the comments raised by the reviewers have been addressed. If you disagree with any of the points raised, please provide adequate justification in your letter. (2) A marked-up version of the manuscript that highlights changes made in response to the reviewers' comments in order to aid the Editors and reviewers. (3) A 'clean' (non-highlighted) version of the manuscript.
- *Reject with the option to resubmit* - In cases where the referees' concerns are very serious and appear unlikely to be addressed within six months, the editor will normally reject the manuscript. If the editor feels the work is of potential interest to the journal, however, they may express interest in seeing a future resubmission. The resubmitted manuscript may be sent back to the original referees or to new referees, at the editor's discretion. If the authors decide to resubmit, the updated version of the manuscript must be submitted online as a new manuscript and should be accompanied by a cover letter that includes a point-by-point response to referees' comments and an explanation of how the manuscript has been changed.
- *Reject outright* - Typically on grounds of specialist interest, lack of novelty, insufficient conceptual advance or major technical and/or interpretational problem

POST-ACCEPTANCE

Once a manuscript is accepted, the corresponding author must complete and sign a Licence to Publish form on behalf of all authors and return it to the editorial office. Failure to promptly return the form will result in delay of publication.
Springer Nature does not require authors of original research papers to assign copyright of their published contributions. Authors grant Springer Nature an exclusive licence to publish, in return for which they can re-use their papers in their future printed work. Springer Nature's author licence page provides details of the policy.

Standard Publication

Manuscripts published under the standard method of publication will be behind a paywall. Readers will be able to access manuscripts through their institutional or personal subscriptions or on a pay-per-view basis. Please click here for a copy of the standard Licence to Publish form.
Government employees from the United States and the UK are required to sign and submit the relevant form below:
- US Government Employee Licence to Publish form
- UK Government Employee Licence to Publish form

Open Access Publication (gold open access)

Upon acceptance, authors can indicate whether they wish to pay an optional article processing charge (APC) for their article to be made open access online immediately upon publication. Open access articles are published under Creative Commons licenses, which allow authors to retain copyright to their work while making it open to readers.
The cost for open access publication in *Oncogene is* £2,500/ $3,300/ €2,700 (VAT or local taxes will be added where applicable).

To facilitate self-archiving Springer Nature deposits open access articles in PubMed Central and Europe PubMed Central on publication. Authors are also permitted to post the final, published PDF of their article on a website, institutional repository or other free public server, immediately on publication.
Visit our open research site for further information about licenses, APCs, and our free OA funding support service:

- About Creative Commons licensing
- Creative Commons license options and article processing charges (APCs) for *Oncogene*
- APC payment FAQs
- Help in identifying funding for APCs
- Editorial process for OA publication in hybrid journals
- Self-archiving and deposition of papers published OA

If authors opt to publish via the open access route then the corresponding author must complete and sign the Article Processing Charge (APC) payment form and an open access License to Publish (LTP) form on behalf of all authors, and return these to the editorial office. These forms will be provided upon acceptance of the article. Failure to promptly return forms will result in delay of publication.

Government employees from the United States and the UK who wish to publish open access are required to sign and submit the relevant form below:
- US Government Employee open access Licence to Publish form
- UK Government Employee open access Licence to Publish form.

Please note with regards to payment that usual credit terms are 30 days from receipt of invoice. Failure to pay your invoice within the stated credit term may result in the Open Access status of the paper being rescinded, with the paper being placed behind the paywall. You may also be subject to such penalties as restrictions on your ability to publish with Springer Nature in the future, involvement of a third party debt collection agency and legal proceedings.

Compliance with open access mandates

Springer Nature's open access journals allow authors to comply with all funders' open access policies worldwide. Authors may need to take specific actions to achieve compliance with funder and institutional open access mandates.
Learn more about open access compliance.

Waiver of institutional open access policies
Please note that Harvard University FAS, MIT, Princeton, UCSF, University of Hawaii at Manoa, California Institute of Technology (Caltech) and the Georgia Institute of Technology have enacted Open Access policies that conflict with our own policy for articles published via the subscription route. If any corresponding or contributing authors are from these institutions, you will need to provide a waiver from the institution of every affected author, which can be obtained from the institution. This waiver should be submitted at the same time as the Licence to Publish form. This requirement does not apply to articles published via the open access route.

Self-archiving and manuscript deposition (green open access)
Authors of original research articles are encouraged to submit the author's version of the accepted paper (the unedited manuscript) to a repository for public release six months after publication. Springer Nature also offers a free, opt-in Manuscript Deposition Service for original research articles in order to help authors fulfil funder and institutional mandates.
Learn more about self-archiving and manuscript deposition

E-Proofs
The Springer Nature e-proofing system is a unique solution that will enable authors to remotely edit /correct your article proofs.
The corresponding author will receive an e-mail containing a URL linking to the e-proofing site. Proof corrections must be returned within 48 hours of receipt. Failure to do so may result in delayed publication. Extensive corrections cannot be made at this stage.
For more information and instructions on how to use the e-proofing too please see here.

Page Charges
After final layout for publication, each page of an article will incur a fixed charge of £153/ $236 per page.
This charge is fully inclusive of colour reproduction of all colour images (where deemed appropriate by the Editor) in print, HTML and PDF formats. It covers also a proportion of the costs of processing and producing the article for publication (VAT or local taxes will be added where applicable).
Page charges will NOT apply to authors who choose to pay an article processing charge to make their paper open access.

Advance Online Publication
The final version of the manuscript is published online in advance of print. AOP represents the official version of the manuscript and will subsequently appear unchanged, in print.

Protocol Exchange
If your manuscript is accepted for publication, we encourage you to upload the step-by-step protocols used in your manuscript to the Protocol Exchange. Protocol Exchange is an open online resource that allows researchers to share their detailed experimental know-how. All uploaded protocols are made freely available, assigned DOIs for ease of citation and fully searchable through nature.com. Protocols can be linked to any publications in which they are used and will be linked to from your article. You can also establish a dedicated page to collect your entire lab Protocols. By uploading your Protocols to Protocol Exchange, you are enabling researchers to more readily reproduce or adapt the methodology you use, as well as increasing the visibility of your protocols and papers. Upload your protocols at the Protocol Exchange web site. Further information can be found here.

Content Sharing
In order to aid the dissemination of research swiftly and legally to the broader community, we are providing all authors with the ability to generate a unique shareable link that will allow anyone to read the published article. If you have selected an Open Access option for your paper, or where an individual can view content via a personal or institutional subscription, recipients of the link will also be able to download and print the PDF.
As soon as your article is published, you can generate your shareable link by entering the DOI of your article here: http://authors.springernature.com/share
We encourage you to forward this link to your co-authors, as sharing your paper is a great way to improve the visibility of your work. There are no restrictions on the number of people you may share this link with, how many times they can view the linked article or where you can post the link online. More information on Springer Nature's commitment to content sharing is available here

EDITORIAL POLICIES

Researchers should conduct their research – from research proposal to publication – in line with best practices and codes of conduct of relevant professional bodies and/or national and international regulatory bodies.

Springer Nature is committed to upholding the integrity of the scientific record. As a member of the Committee on Publication Ethics (COPE), *Oncogene* abides by COPE's principles on how to deal with potential acts of misconduct, which includes formal investigation of all perceived transgressions.

Authorship
Requirements for all categories of articles should conform to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals," developed by the ICMJE (www.icmje.org).

Each author must have contributed sufficiently to the intellectual content of the submission. The corresponding author should list all authors and their contributions to the work. The corresponding author must confirm that he or she has had full access to the data in the study and final responsibility for the decision to submit for publication.

To qualify as a contributing author, one must meet all of the following criteria:
1. Conceived and/or designed the work that led to the submission, acquired data, and/or played an important role in interpreting the results.
2. Drafted or revised the manuscript.

3. Approved the final version.
4. Agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Contributions by individuals who made direct contributions to the work but do not meet all of the above criteria should be noted in the Acknowledgments section of the manuscript. Medical writers and industry employees can be contributors. Their roles, affiliations, and potential conflicts of interest should be included in the author list or noted in the Acknowledgments and/or Contributors section concurrent with their contribution to the work submitted. Signed statements from any medical writers or editors declaring that they have given permission to be named as an author, as a contributor, or in the Acknowledgments section is also required. Failure to acknowledge these contributors can be considered inappropriate, which conflicts with the journal's editorial policy.

Changes to authorship

It is the corresponding author's responsibility to ensure that the author list is correct at the point of first submission. Requests to change the authorship (such as to include or exclude an author, change an author's name or contribution) must be accompanied by a letter signed by all authors to show they concur with the change. New authors must also confirm that they fully comply with the journal's authorship requirements. Requests for addition or removal of authors as a result of authorship disputes (after acceptance) are honoured after formal notification by the institute or independent body and/or when there is agreement between all authors. Changes to the authorship will not be allowed once the manuscript has been accepted for publication.

Correspondence with the Journal

One author is designated the contact author for matters arising from the manuscript (materials requests, technical comments and so on). It is this author's responsibility to inform all co-authors of matters arising and to ensure such matters are dealt with promptly. Before submission, the corresponding author ensures that all authors are included in the author list, its order agreed upon by all authors, and are aware that the manuscript was submitted. After acceptance for publication, proofs are e-mailed to this corresponding author who should circulate the proof to all co-authors and coordinate corrections among them

Anonymity and Confidentiality

Editors, authors and reviewers are required to keep confidential all details of the editorial and peer review process on submitted manuscripts. Unless otherwise declared as a part of open peer review, the peer review process is confidential and conducted anonymously. All details about submitted manuscripts are kept confidential and no comments are issued to outside parties or organizations about manuscripts under consideration or if they are rejected. Editors are restricted to making public comments on a published article's content and their evaluation.

Upon accepting an invitation to evaluate a manuscript, reviewers must keep the manuscript and associated data confidential, and not redistribute them without the journal's permission. If a reviewer asks a colleague to assist in assessing a manuscript, confidentiality must be ensured and their names must be provided to the journal with the final report.

We ask reviewers not to identify themselves to authors without the editor's knowledge. If they wish to reveal their identities while the manuscript is under consideration, this should be done via the editor; if this is not practicable, we ask authors to inform the editor as soon as possible after the reviewer has revealed their identity. Our own policy is to neither confirm nor deny any speculation about reviewers' identities, and we encourage reviewers to adopt a similar policy.

We deplore any attempt by authors to confront reviewers or try to determine their identities. Reviewers should be aware that it is our policy to keep their names confidential and that we do our utmost to ensure this confidentiality. We cannot, however, guarantee to maintain this confidentiality in the face of a successful legal action to disclose identity.

Regardless of whether a submitted manuscript is eventually published, correspondence with the journal, referees' reports, and other confidential material must not be published, disclosed, or otherwise publicised without prior written consent.

Communication with the Media

Material submitted must not be discussed with the media. We reserve the right to halt the consideration or publication of a paper if this condition is broken. If a paper is particularly newsworthy, the press release will be sent to our list of journalists in advance of publication with an embargo that forbids any coverage of the manuscript, or the findings of the manuscript, until the time and date clearly stated. Authors whose papers are scheduled for publication may also arrange their own publicity (for instance through their institution's press offices), but they must strictly adhere to our press embargo and are advised to coordinate their own publicity with our press office.

Pre- and Post-Submissions

Authors are welcome to post pre-submission versions or the original submitted version of the manuscript on a personal blog, a collaborative wiki or a recognized preprint server (such as ArXiv or BioRXiv) at any time (but not subsequent pre-accept versions that evolve following peer review).
For subscribed content, the accepted version of the manuscript, following the review process, may only be posted 6 months after the paper is published in a Springer Nature journal. A publication reference and URL to the published version on the journal website must be provided on the first page of the postprint. The published version — copyedited and in the individual Springer Nature journal format — may not be posted on any website or preprint server.
For open access content published under a creative commons license, authors can replace the submitted version with the final published version at publication as long as a publication reference and URL to the published version on the journal website are provided

Permissions

If a table or figure has been published before, the authors must obtain written permission to reproduce the material in both print and electronic formats from the copyright owner and submit it with the manuscript. This follows for illustrations and other materials taken from previously published works not in the public domain. The original source should be cited in the figure caption or table footnote. Permission to reproduce material can usually be obtained through the Copyright Clearance Center.

Competing Interests

In the interests of transparency and to help readers form their own judgments of potential bias, authors must declare whether or not there are any competing financial interests in relation to the work described. The corresponding author is responsible for submitting a competing financial interests statement on behalf of all authors of the paper. This statement must be included in the cover letter and on the title page of the manuscript, as well as within the article before the References section listed under 'Competing Interests'.
In cases where the authors declare a competing financial interest, a statement to that effect is published as part of the article. If no such conflict exists, the statement will simply read that the authors have nothing to disclose.
For the purposes of this statement, competing interests are defined as those of a financial nature that, through their potential influence on behaviour or content, or from perception of such potential influences, could undermine the objectivity, integrity or perceived value of a publication. They can include any of the following:

- *Funding:* Research support (including salaries, equipment, supplies, reimbursement for attending symposia, and other expenses) by organizations that may gain or lose financially through this publication. The role of the funding body in the design of the study, collection and analysis of data and decision to publish should be stated.
- *Employment:* Recent (while engaged in the research project), present or anticipated employment by any organization that may gain or lose financially through this publication. This includes positions on an advisory board, board of directors, or other type of management relationship.
- *Personal financial interests:* Stocks or shares in companies that may gain or lose financially through publication; consultation fees or other forms of remuneration from organisations that may gain or lose financially.
- *Patents:* Holding, or currently applying for, patents, relating to the content of a manuscript; receiving reimbursement, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript.

It is difficult to specify a threshold at which a financial interest becomes significant, but note that many US universities require faculty members to disclose interests exceeding $10,000 or 5% equity in a company. Any such figure is arbitrary, so we offer as one possible practical alternative guideline: "Declare all interests that could embarrass you were they to become publicly known after your work was published." We do not consider diversified mutual funds or investment trusts to constitute a competing financial interest.

The statement included in the submission must contain an explicit and unambiguous description of any potential competing interests, or lack thereof, for any of the authors as it relates to the subject of the report. Examples include:

- Competing Interests
  The authors declare no competing financial interests
- Competing Interests
  Dr Caron's work has been funded by the NIH. He has received compensation as a member of the scientific advisory board of Acadia Pharmaceutical and owns stock in the company. He also has consulted for Lundbeck and received compensation. Dr Rothman and Dr Jensen declare no potential competing interests.

Neither the precise amount received from each entity nor the aggregate income from these sources needs to be provided.

Non-financial interests that authors may like to disclose include:

- a close relationship with, or a strong antipathy to, a person whose interests may be affected by publication of the article,
- an academic link or rivalry with someone whose interests may be affected by publication of the article,
- membership in a political party or special interest group whose interests may be affected by publication of the article, or
- a deep personal or religious conviction that may have affected what the author wrote and that readers should be aware of when reading the article.

Studies involving animals and other human subjects

Research involving human subjects, human material, or human data must have been performed in accordance with the Declaration of Helsinki and must have been approved by an appropriate ethics committee. A statement detailing this, including the name of the ethics committee and the reference number where appropriate, along with a statement confirming that informed consent was obtained from all subjects, must appear in all manuscripts reporting such research.

For primary research manuscripts reporting experiments on live vertebrates and/or higher invertebrates, the corresponding author must confirm that all experiments were performed in accordance with relevant guidelines and regulations. The manuscript must include in the Supplementary Information (methods) section (or, if brief, within of the print/online article at an appropriate place), a statement identifying the institutional and/or licensing committee approving the experiments, including any relevant details regarding animal welfare, patient anonymity, drug side effects and informed consent. Sex and other characteristics of animals that may influence results must be described. Details of housing and husbandry must be included where they are likely to influence experimental results. *Oncogene* recommends following the ARRIVE reporting guidelines when documenting animal studies.

Clinical Trials

All clinical trials must be registered in a public registry prior to submission and the trial registry number must be included in the manuscript and provided on submission.. The journal follows the trials registration policy of the ICMJE (www.icmje.org) and considers only trials that have been appropriately registered before submission, regardless of when the trial closed to enrolment. Acceptable registries must meet the following ICMJE requirements:

- be publicly available, searchable, and open to all prospective registrants
- have a validation mechanism for registration data
- be managed by a not-for-profit organization

Examples of registries that meet these criteria include:

1. ClinicalTrials.gov the registry sponsored by the United States National Library of Medicine
2. the International Standard Randomized Controlled Trial Number Registry
3. the Cochrane Renal Group Registry
4. the European Clinical Trials Database

Randomised Controlled Trials (RCTs) must adhere to the CONSORT statement, (CONsolidated Standards Of Reporting Trials) and submissions must be accompanied by a completed CONSORT checklist (uploaded as a related manuscript file). Further information can be found at www.consort-statement.org.

Informed Consent

When publishing identifiable images from human research participants, authors must include a statement attesting that they have obtained informed consent for publication of the images. If the participant is deceased, consent must be sought from the next of kin of the participant. All reasonable measures must be taken to protect patient anonymity. Black bars over the eyes are not acceptable means of anonymization. In certain cases, the journal may insist upon obtaining evidence of informed consent from authors. Images without appropriate consent will be removed from publication.

Cell Line Authentication

If human cell lines are used, authors are strongly encouraged to include the following information in their manuscript:

- the source of the cell line, including when and from where it was obtained,
- whether the cell line has recently been authenticated and by what method, and
- whether the cell line has recently been tested for mycoplasma contamination.

Further information is available from the International Cell Line Authentication Committee (ICLAC). We recommend that authors check the NCBI database for misidentification and contamination of human cell lines.

Biosecurity Policy

The Editor may seek advice about submitted papers not only from technical reviewers but also on any aspect of a paper that raises concerns. These may include, for example, ethical issues or issues of data or materials access. Occasionally, concerns may also relate to the implications to society of publishing a paper, including threats to security. In such circumstances, advice will usually be sought simultaneously with the technical peer-review process. As in all publishing decisions, the ultimate decision whether to publish is the responsibility of the editor.

Reproducibility

Oncogene requires authors of papers that are sent for external review to include in their manuscripts relevant details about several elements of experimental and analytical design. This initiative aims to improve the transparency of reporting and the reproducibility of published results, focusing on elements of methodological information that are frequently poorly reported. Authors being asked to resubmit a manuscript will be asked to confirm that these elements are included by filling out a checklist that will be made available to the editor and reviewers.

Research Data Policy

An inherent principle of publication is that others should be able to replicate and build upon the authors' published claims. We strongly encourage that all datasets on which the conclusions of the paper rely should be available to readers. We encourage authors to ensure that their datasets are either deposited in publicly available repositories (where available and appropriate) or presented in the main manuscript or additional supporting files whenever possible. If a public repository does not exist, the information must be made available to editors and referees at submission, and to readers promptly upon request. Any restrictions on material availability or other relevant information must be disclosed in the manuscript's Methods section and should include details of how materials and information may be obtained.
Please see the journals guidelines on Research Data policy here.

Sequences, Structures and "Omics"

Papers reporting protein or DNA sequences and molecular structures will not be accepted without an accession number to Genbank, EMBL, DDBJ, Uniprot, ProteinDataBank, or other publicly available database in general use in the field that gives free access to researchers from the date of publication.

Authors of papers describing structures of biological macromolecules must provide experimental data upon the request of Editor if they are not already freely accessible in a publicly available database such as ProteinDataBank, Biological Magnetic Resonance Databank, or Nucleic Acid Database.

Misconduct

Springer Nature takes seriously all allegations of potential misconduct. As a member of the Committee on Publication Ethics (COPE), *Oncogene* will follow the COPE guidelines outlining how to deal with cases of suspected misconduct. As part of the investigation, the journal may opt to do one or more of the following:

- suspend review or publication of a paper until the issue has been investigated and resolved;

- request additional information from the author, including original data or images or ethics committee or IRB approval;
- make inquiries of other titles believed to be affected;
- forward concerns to the author's employer or person responsible for research governance at the author's institution;
- refer the matter to other authorities or regulatory bodies (for example, the Office of Research Integrity in the US or the General Medical Council in the UK); or
- submit the case to COPE in an anonymized form for additional guidance on resolution.

Please note that, in keeping with the journal's policy of the confidentiality of peer review, if sharing of information with third parties is necessary, disclosure will be made to only those Editors who the Editor believes may have information that is pertinent to the case, and the amount of information will be limited to the minimum required.

Duplicate Publication

Papers must be original and not published or submitted for publication elsewhere. This rule also applies to non-English language publications..
Springer Nature allows and encourages prior publication on recognized community preprint servers for review by other scientists before formal submission to a journal. The details of the preprint server concerned and any accession numbers should be included in the cover letter accompanying manuscript submission. This policy does not extend to preprints available to the media or that are otherwise publicized outside the scientific community before or during the submission and consideration process.
Springer Nature also allows publication of meeting abstracts before the full contribution is submitted. Such abstracts should be included with the journal submission and referred to in the cover letter accompanying the manuscript. Again this policy does not extend to meeting abstracts and reports available to the media or which are otherwise publicised outside the scientific community during the submission and consideration process.

Plagiarism

Plagiarism is when an author attempts to pass off someone else's work as his or her own. Duplicate publication, sometimes called self-plagiarism, occurs when an author reuses substantial parts of his or her own published work without providing the appropriate references. This can range from getting an identical paper published in multiple journals, to 'salami-slicing', where authors add small amounts of new data to a previous paper.

Plagiarism can be said to have clearly occurred when large chunks of text have been cut-and-pasted. Minor plagiarism without dishonest intent is relatively frequent, for example, when an author reuses parts of an introduction from an earlier paper. Journal editors judge any case of which they become aware (either by their own knowledge of and reading about the literature, or when alerted by referees) on its own merits.
Springer Nature is a member of Similarity Check (formerly CrossCheck), a multi-publisher initiative used to screen published and submitted content for originality. *Oncogene* uses Similarity Check to detect instances of overlapping and similar text in submitted manuscripts. To find out more about visit the Similarity Check website.

If a case of plagiarism comes to light after a paper is published, the Journal will conduct a preliminary investigation, utilising the guidelines of the Committee on Publication Ethics. If plagiarism is proven, the Journal will contact the author's institute and funding agencies as appropriate. The paper containing the plagiarism may also be formally retracted or subject to correction.

Data Fabrication & Falsification

Falsification is the practice of altering research data with the intention of giving a false impression. This includes, but is not limited to, manipulating images, removing outliers or "inconvenient" results, or changing, adding or omitting data points. Fabrication is the practice of inventing data or results and recording and/or reporting them in the research record. Data falsification and fabrication call into question the integrity and credibility of data and the data record, and as such, they are among the most serious issues in scientific ethics.
Some manipulation of images is allowed to improve them for readability. Proper technical manipulation includes adjusting the contrast and/or brightness or colour balance if it is applied to the complete digital image (not parts of the image). The author should notify the Editor in the cover letter of any technical manipulation. Improper technical manipulation refers to obscuring, enhancing, deleting and/or introducing new elements into an image. See Image Integrity & Standards below for more details.

Image Integrity and Standards

Images submitted with a manuscript for review should be minimally processed (for instance, to add arrows to a micrograph). Authors should retain their unprocessed data and metadata files, as editors may request them to aid in manuscript evaluation. If unprocessed data is unavailable, manuscript evaluation may be stalled until the issue is resolved.

A certain degree of image processing is acceptable for publication, but the final image must correctly represent the original data and conform to community standards. The guidelines below will aid in accurate data presentation at the image processing level:

- Authors should list all image acquisition tools and image processing software packages used. Authors should document key image-gathering settings and processing manipulations in the Methods section.
- Images gathered at different times or from different locations should not be combined into a single image, unless it is stated that the resultant image is a product of time-averaged data or a time-lapse sequence. If juxtaposing images is essential, the borders should be clearly demarcated in the figure and described in the legend.
- Touch-up tools, such as cloning and healing tools in Photoshop, or any feature that deliberately obscures manipulations, is to be avoided.
- Processing (such as changing brightness and contrast) is appropriate only when it is applied equally across the entire image and is applied equally to controls. Contrast should not be adjusted so that data disappear. Excessive manipulations, such as processing to emphasize one region in the image at the expense of others (for example, through the use of a biased choice of threshold settings), is inappropriate, as is emphasizing experimental data relative to the control.

For **gels and blots**, positive and negative controls, as well as molecular size markers, should be included on each gel and blot – either in the main figure or an expanded data supplementary figure. The display of cropped gels and blots in the main paper is encouraged if it improves the clarity and conciseness of the presentation. In such cases, the cropping must be mentioned in the figure legend.

- Vertically sliced gels that juxtapose lanes that were not contiguous in the experiment must have a clear separation or a black line delineating the boundary between the gels.
- Cropped gels in the paper must retain important bands.
- Cropped blots in the body of the paper should retain at least six band widths above and below the band.
- High-contrast gels and blots are discouraged, as overexposure may mask additional bands. Authors should strive for exposures with gray backgrounds. Immunoblots should be surrounded by a black line to indicate the borders of the blot, if the background is faint.
- For quantitative comparisons, appropriate reagents, controls and imaging methods with linear signal ranges should be used.

Microscopy adjustments should be applied to the entire image. Threshold manipulation, expansion or contraction of signal ranges and the altering of high signals should be avoided. If 'pseudo-colouring' and nonlinear adjustment (for example 'gamma changes') are used, this must be disclosed. Adjustments of individual colour channels are sometimes necessary on 'merged' images, but this should be noted in the figure legend. We encourage inclusion of the following with the final revised version of the manuscript for publication:

- In the Methods section, specify the type of equipment (microscopes/objective lenses, cameras, detectors, filter model and batch number) and acquisition software used. Although we appreciate that there is some variation between instruments, equipment settings for critical measurements should also be listed.
- The display lookup table (LUT) and the quantitative map between the LUT and the bitmap should be provided, especially when rainbow pseudo-colour is used. It should be stated if the LUT is linear and covers the full range of the data.
- Processing software should be named and manipulations indicated (such as type of deconvolution, three-dimensional reconstructions, surface and volume rendering, 'gamma changes', filtering, thresholding and projection).
- Authors should state the measured resolution at which an image was acquired and any downstream processing or averaging that enhances the resolution of the image.

Correction and Retraction Process

If there is suspicion of misconduct, the journal will carry out an investigation following COPE guidelines. Following an investigation, if the allegation raises valid concerns, the author will be contacted and given an opportunity to address the issue. If misconduct is established beyond reasonable doubt, this may result in the Editor implementing one of the following measures:

- If the article is still under consideration, it may be rejected and returned to the author.
- If the article has already been published online, depending on the nature and severity of the infraction, either an erratum will be published alongside the article or, in severe cases, complete retraction of the article will occur. The reason for the erratum or retraction must be given.
- In either case, the author's institution or funding agency may be informed.

Content published as Advance Online Publication (AOP) is final and cannot be amended. The online and print versions are both part of the published record hence the original version must be preserved and changes to the paper should be made as a formal correction. If an error is noticed in an AOP article, a correction should accompany the article when it publishes in print. An HTML (or full-text) version of the correction will also be created and linked to the original article. If the error is found in an article after print publication the correction will be published online and in the next available print issue.

Please note the following categories of corrections to print and online versions of peer reviewed content:

- **Erratum**. Notification of **an important error made by the journal** that affects the publication record or the scientific integrity of the paper, or the reputation of the authors, or of the journal.
- **Corrigendum**. Notification of **an important error made by the author** that affects the publication record or the scientific integrity of the paper, or the reputation of the authors or the journal.
- **Retraction**. Notification of **invalid results**. All co-authors must sign a retraction specifying the error and stating briefly how the conclusions are affected.

Decisions about corrections are made by the Editor (sometimes with peer-reviewers' advice) and this sometimes involves author consultation. Requests to make corrections that do not affect the paper in a significant way or impair the reader's understanding of the contribution (a spelling mistake or grammatical error, for example) are not considered.

In cases where co-authors disagree about a correction, the editors will take advice from independent peer-reviewers and impose the appropriate correction, noting the dissenting author(s) in the text of the published version

FURTHER INFORMATION

For inquiries related to submission requirements, please contact the editorial office. For inquiries related to advertising, subscriptions, permissions, papers in production or publishing a supplement, please contact the publisher's office.