A Chemical Screen Identifies Compounds Capable of Selecting for Haploidy in Mammalian Cells

Highlights

- Mammalian haploid cell cultures become progressively enriched in diploid cells
- DAB, a precursor of Taxol, facilitates the maintenance of haploidy
- DAB selects for cells with lower ploidy in mixed cultures of mammalian cells
- Statins accelerate the gradual loss of haploid cells in culture

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In Brief
Cultures of haploid animal cell lines become progressively enriched in diploid cells. By conducting a chemical screen in HAP1 cells, Olbrich et al. identify compounds that facilitate the maintenance of haploid cells and a general strategy to select for cells with lower ploidy in mixed cultures of mammalian cells.

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A Chemical Screen Identifies Compounds Capable of Selecting for Haploidy in Mammalian Cells

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SUMMARY

The recent availability of somatic haploid cell lines has provided a unique tool for genetic studies in mammals. However, the percentage of haploid cells rapidly decreases in these cell lines, which we recently showed is due to their overgrowth by diploid cells present in the cultures. Based on this property, we have now performed a phenotypic chemical screen in human haploid HAP1 cells aiming to identify compounds that facilitate the maintenance of haploid cells. Our top hit was 10-Deacetyl-baccatin-III (DAB), a chemical precursor in the synthesis of Taxol, which selects for haploid cells in HAP1 and mouse haploid embryonic stem cultures. Interestingly, DAB also enriches for diploid cells in mixed cultures of diploid and tetraploid cells, including in the colon cancer cell line DLD-1, revealing a general strategy for selecting cells with lower ploidy in mixed populations of mammalian cells.

INTRODUCTION

With a few exceptions like wasps or mites, haploidy in animals is confined to the germline (Wutz, 2014). A diploid genome increases genetic diversity and buffers the impact of mutations due to the presence of two copies of each gene. However, this feature has also limited genetic screenings in mammalian cells until recently, which were more efficiently conducted in model organisms that can grow as haploids such as yeast. Accordingly, the isolation of animal haploid somatic cell lines has been something attempted since the 1960s with initial efforts focusing on frogs and insects (Debec, 1978; Freed, 1982; Freed and Mezger-Freed, 1970). Through parthenogenesis (triggering development of unfertilized oocytes) or androgenesis (development of zygotes only containing a paternal genome), haploid mammalian embryonic stem cells (haESCs) have now been successfully derived from a wide range of species including zebrafish, mouse, rat, pig, monkey, and humans (Elling et al., 2011; Leeb and Wutz, 2011; Sagi et al., 2016; Yang et al., 2013; Yi et al., 2009). In addition to haESCs, a cell line (KBM7) harboring a near-haploid genome except for two copies of chromosome 8 was isolated from a leukemia patient (Andersson et al., 1995). KBM7 cells were subsequently used for the generation of an adherent derivative (HAP1) that has lost one copy of chromosome 8 and is thus virtually haploid (Carette et al., 2011). As expected, haploid mammalian cell lines rapidly became a powerful tool for forward genetic screenings and have been successfully used in many such approaches (Blomen et al., 2015; Carette et al., 2011; Jae et al., 2013, 2014; Papatheodorou et al., 2011; Rong et al., 2015; Wang et al., 2015).

Despite the usefulness of haploid cell lines, haploidy is an unstable state in mammalian somatic cells as these cultures rapidly become enriched in diploids, which demands frequent sorting in order to maintain a good percentage of haploid cells (reviewed in Yilmaz et al., 2016). While this phenomenon was originally thought to be a consequence of diploidization, we recently showed that it is caused by the activation of a p53-dependent response, which limits the viability of the haploid cells that are progressively overgrown by diploid cells present in the cultures (Olbrich et al., 2017). Accordingly, p53 deficiency facilitates the maintenance of haploidy in mouse haESCs (mhaESCs) or HAP1 cells. Besides the identification of genetic conditions that help to stabilize the haploid state, chemical strategies could also facilitate the maintenance of haploid cultures. While some efforts in this area have been made, they focused on a few selected compounds and came to opposing conclusions, as either activating (Takahashi et al., 2014) or inhibiting (He et al., 2017) CDK1 was reported to stabilize haploidy in mhaESCs. We here report on a chemical screen to identify compounds capable of facilitating the maintenance of haploid cells, both in mhaESCs and in HAP1 cells, and that has revealed a general
strategy to select for cells with lower ploidy in mixed cultures of mammalian cells.

RESULTS AND DISCUSSION

A Chemical Screen for Drugs That Stabilize Haploidy in HAP1 Cells

As mentioned above, we recently identified that the progressive reduction in the percentage of haploid cells observed in haploid mammalian cell lines is due to their outgrowth by diploid cells existing in these cultures (Olbrich et al., 2017). Based on this property, and in order to identify chemicals that facilitate the maintenance of haploidy, we performed a chemical screen in HAP1 cells using a library of 977 bioactive compounds, including many US Food and Drug Administration (FDA)-approved agents. To do so, haploid and diploid HAP1 cells were isolated by fluorescence-activated cell sorting (FACS) and subsequently infected with lentiviruses encoding for tdTomato fluorescence protein (TOM) or EGFP, respectively. HaploidTOM and diploidEGFP populations of HAP1 cells were then mixed at a 4:1 ratio, seeded in 96-well plates, and treated individually with the chemicals from the library for 3 weeks (Figure 1A). During the screen, compounds were added twice per week and cells were split once per week. At the endpoint of the experiment, the percentage of haploid and diploid HAP1 cells was quantified by high-throughput flow cytometry on the basis of EGFP- and TOM-positive cell fractions.

The results of the screen were plotted as the percentage of haploid cells observed in the wells treated with compounds normalized to that observed in control wells (dimethylsulfoxide [DMSO]). This representation followed a normal distribution, with several compounds showing a clear impact on the percentage of TOM-positive HAP1 cells remaining after the 3-week period (Figure 1B). From the initial screen, we selected 37 compounds that either increased (n = 19) or decreased (n = 18) the percentage of haploid cells more than 30% from that observed in control (DMSO treated) wells. The panel represents the data from 930 compounds, as 47 chemicals killed all cells during the experiment (see Table S1).

(B) Graph representing the results of the screen defined in (A), indicating the thresholds at which compounds either increased or decreased the percentage of haploidy in more than 30% from that observed in control (DMSO treated) wells. The panel represents the data from 930 compounds, as 47 chemicals killed all cells during the experiment (see Table S1).

(C) Flow cytometry analysis of DNA content in HAP1 cells that were sorted for haploidy by FACS on day 0 and after 26 days of growth in the presence of DMSO (control), DAB (10 μM), or Diclazuril (10 μM). The fraction of haploid G1 cells is highlighted in red. One representative example out of three independent experiments is shown.

(D) Flow cytometry analysis of DNA content in HAP1 cells that were sorted for haploidy by FACS on day 0 and after 19 days of growth in the presence of DMSO (control), Mevastatin (10 μM), Atorvastatin (10 μM), Lovastatin (10 μM), Fluvastatin (10 μM), or Simvastatin (10 μM). The fraction of haploid G1 cells is highlighted in red. One representative example out of three independent experiments is shown.

10-Deacetylbaccatin-III Selects for Haploid Cells in HAP1 and mhaESCs

The two compounds that consistently stabilized haploidy in HAP1 cells in validation experiments were 10-Deacetylbaccatin-III (DAB), a natural compound isolated from the yew tree...
and that is used as a precursor in the synthesis of paclitaxel (Guér ifte-Vegelein et al., 1986), and Diclazuril, an antiparasitic used in the prevention of bovine coccidiosis (Zechner et al., 2015). However, while DAB was able to stabilize haploidy both in HAP1 as well as in mhaESCs (see below), the effects of Diclazuril were restricted to HAP1 cells. Thus, we selected DAB for mechanistic studies. Of note, we also observed mild effects in stabilizing haploidy in HAP1 cells with other compounds such as the anticancer kinase inhibitors Regorafenib or Nilotinib (Figure S1). However, these compounds overall decrease growth rates, which would simply slow down the overgrowth of haploid cells by diploids.

To further define the potency of DAB in stabilizing haploidy in mammalian cultures, we analyzed its effects in HAP1 cultures where haploid cells were a minority within the mixed population. To do so, haploidTOM and diploidEGFP HAP1 cells were mixed in a 1:4 ratio and grown in the presence or absence of DAB (Figure 2A). By 18 days, the population of haploidTOM cells in control conditions had been reduced to 3% from the culture. Strikingly, when the mixed culture was grown in the presence of DAB, the population of haploid HAP1 cells not only did not decrease but actually increased to 45% by day 18. We next evaluated the effect of DAB in the growth of mhaESCs, where the progressive loss of haploid cells occurs significantly faster than in HAP1 cells (Olbrich et al., 2017). Accordingly, in 2 weeks of culture the haploid cells had almost disappeared from mhaESC that was sorted for haploidy by FACS on day 0 and after 14 days of growth in the presence of DMSO (control) or DAB (5 μM). The fraction of haploid G1 cells is highlighted in red. One representative example out of two different experiments made in independent mhaESC clones is shown.

DAB Selects for Lower Ploidy in Mixed Cultures of Mammalian Cells

Given that DAB selects for cells with a 1n DNA content in mixed cultures of haploid and diploid cells, we hypothesized that this could be a general property of the chemical to select for cells with lower ploidy in mixed cultures of mammalian cells. To do so, we first isolated a tetraploid population from HAP1 cultures by FACS and evaluated the effects of DAB in these cells. Consistent with our hypothesis, tetraploid HAP1 cells had nearly disappeared after 20 days of culture in the presence of DAB and were replaced by diploids, which were likely contaminants carried over during the isolation of the tetraploid fraction (Figure 3A).

To further test the effects of DAB in selecting for lower ploidy, we infected tetraploid HAP1 cells with lentiviruses expressing blue fluorescent protein (BFP; tetraploidBFP) and mixed them at 1:1:1 ratio with haploidTOM and diploidEGFP HAP1 cells. In agreement with the reduced fitness of mammalian haploid and tetraploid cells (Olbrich et al., 2017; Pfau et al., 2016), both populations were progressively overgrown by diploidEGFP HAP1 cells (Figure 3B). In contrast, when the mixed cultures were grown in the presence of DAB, these became increasingly enriched in haploidTOM cells, followed by diploidEGFP and finally by tetraploidBFP cells, confirming a general effect of DAB in selecting for cells with lower ploidy.
In which mammalian cells, we evaluated the effects of DAB in HAP1 cells. First, given that p53 deficiency stabilizes haploidy in mammalian cells, including cancer cells.

Last, we sought to determine the mechanism by which DAB selects for cells with lower ploidy in mixed cultures of mammalian cells. To do so, we evaluated the effects of DAB in mouse embryonic fibroblasts (MEFs), which have a tendency to spontaneously tetraploidize. Cultures of diploid MEF expressing EFGP and tetraploid cells expressing tdTomato at a 1:4 ratio were grown in the presence of DAB (10 μM) for 13 days. While, consistent with the reduced fitness of tetraploid mammalian cells (Pfau et al., 2016), the tetraploid diploid MEF population had decreased from 80% to 52% by day 13, this nearly disappeared if the cultures were grown in the presence of DAB (Figure 3C). Finally, we evaluated if these findings were also conserved in cancer cells, as tetraploidy or absence of DAB. While, in agreement with the reduced fitness of tetraploid mammalian cells (Pfau et al., 2016), the tetraploid diploid MEF population had decreased from 80% to 52% by day 13, this nearly disappeared if the cultures were grown in the presence of DAB (Figure 3C).

The ploidy-dependent toxicity of DAB provides a mechanism to explain its effects on selecting for cells with lower ploidy in mixed cultures of mammalian cells. To determine if the effects of DAB were not restricted to HAP1 cells, we evaluated its effects in mouse embryonic fibroblasts (MEFs), which have a tendency to spontaneously tetraploidize. Cultures of diploid MEF expressing EFGP and tetraploid cells expressing tdTomato at a 1:4 ratio were grown in the presence of DAB (10 μM) for 13 days. While, consistent with the reduced fitness of tetraploid mammalian cells (Pfau et al., 2016), the tetraploid diploid MEF population had decreased from 80% to 52% by day 13, this nearly disappeared if the cultures were grown in the presence of DAB (Figure 3C).

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DAB Promotes Mitotic Arrest in a Ploidy-Dependent Manner

Last, we sought to determine the mechanism by which DAB works. First, given that p53 deficiency stabilizes haploidy in mammalian cells, we evaluated the effects of DAB in HAP1 cells in which P53 had been deleted by CRISPR-Cas9. However, DAB was able to increase the fraction of haploid cells both in wild-type and P53-deficient HAP1 cells upon 25 days in culture (Figure S3A). Next, given that DAB is a precursor in the synthesis of paclitaxel, which stabilizes microtubules by preventing their disassembly, we explored if DAB could have similar effects. In fact, evaluation of the intracellular distribution of α-tubulin after microtubule depolymerization induced by a cold shock revealed a clear effect of DAB in the microtubule dynamics of interphase cells (Figure S3B). Since microtubule reorganization is particularly relevant for the assembly of the mitotic spindle, we then evaluated the effects of DAB on the time that cells spend in mitosis. To do so, we infected haploid, diploid, and tetraploid HAP1 cells with a histone H2B-red fluorescent protein (RFP) fusion expressing lentivirus and monitored the effect of DAB in these cell lines by live-cell video-microscopy (Figure 4A). These analyses revealed that DAB extended the duration of mitosis in all three cell lines, with the severity of the arrest correlating with their ploidy (Figures 4A and 4B).

Importantly, while most haploid cells could overcome the mitotic arrest induced by DAB and continue cell division, diploids and particularly tetraploid HAP1 cells presented very prolonged arrests that were often followed by cell death. Flow cytometry analyses of DNA content confirmed the ploidy-dependent toxic effects of DAB in HAP1 cells (Figure S4A). Accordingly, while DAB did not significantly affect the growth of haploid HAP1 cells, it had a higher impact on diploid and particularly tetraploid HAP1 cultures (Figure S4B). The ploidy-dependent toxicity of DAB provides a mechanism to explain its effects on selecting for cells with lower ploidy in mixed cultures of mammalian cells.

Further analyses of the images from the video microscopy experiment revealed that the extended duration of mitosis induced...
by DAB was mainly due to an effect on the compound in delaying the formation of a metaphase plate (Figure 4A). Accordingly, while immunofluorescence analyses revealed normal metaphase and anaphase figures in haploid HAP1 cells treated with DAB, mitoses from diploids and even more so from tetraploids revealed that these were arrested at pro-metaphase with a high proportion of...
lagging chromosomes (Figure S4C). To further analyze the effects of DAB in impairing mitotic progression, we used a U2OS cell line stably expressing a cyclin B-mCherry fusion (Gavet and Pines, 2010). Since cyclin B levels are highest at mitotic entry and lowest at the onset of anaphase (Clute and Pines, 1999), this system can be used to quantify the time needed to assemble a complete metaphase plate. These analyses confirmed the effects of DAB in mitosis, in a manner comparable to those that can be achieved with low doses of paclitaxel (10 nM) (Figure 4C). Based on these findings, we hypothesized that low doses of paclitaxel could also be used to select for cells with lower ploidy. Indeed, a low dose of paclitaxel selected for haploid cells in mixed cultures of haploid and diploid HAP1 cells, and for diploid cells in mixed cultures of diploid and tetraploid DLD-1 cells (Figures 4D and 4E).

We here present our work on a chemical screen to identify chemicals that facilitate the maintenance of haploidy in mammalian cells. The paclitaxel precursor DAB fulfills this objective, providing a useful and facile manner to overcome the progressive loss of haploidy in mammalian haploid cultures. While other compounds such as Regorafenib or Nilotinib could also moderately diminish diploidization rates, these are drugs that reduce cellular growth rates in multiple cell types, so that their effects can be explained by a slowdown in the overgrowth of haploid cells by diploids, which we recently showed is the basis of the observed diploidization in HAP1 cells and mhaESCs (Olbrich et al., 2017). Accordingly, “diploidization” rates are much faster in haploid ESCs than in HAP1 or KBM7 cells due to the faster proliferation of stem cell cultures. Strategies that select for haploid cells, chemical or genetic, should facilitate further research in the biology of haploid mammalian cells, and might enable us to overcome some of the remaining challenges such as the generation of additional primary differentiated haploid cell lines different to ESCs. Of note, and while we here focused on compounds that stabilize haploidy, it is remarkable that all chemicals having an opposite effect, namely that they accelerate the loss of haploid cells, were statins, broadly used to lower cholesterol levels in humans. Even if at this point we lack an understanding for this observation, it is interesting that recent studies have identified a particular dependency of aneuploid cells on the biosynthesis of sphingolipids (Hwang et al., 2017; Tang et al., 2017). To what extent lipid biosynthesis is particularly relevant for cells with an altered ploidy deserves further attention.

In regard to the mechanism of action of DAB, this compound is normally used as a precursor in the synthesis of paclitaxel and was not known to have biological activity. In fact, and while the compound was included as part of an FDA-approved library, it is not approved per se and has no medical use. Nevertheless, we here show that DAB, while significantly less potent than paclitaxel, does have effects on microtubule dynamics and mitotic progression. Of note, while we previously observed that mhaESCs are more sensitive than diploid mESCs to high doses of paclitaxel (Olbrich et al., 2017), it is well documented that the suppression of microtubule dynamics with paclitaxel occurs at much lower concentrations than doses needed to exert toxicity (Jordan et al., 1993). Accordingly, the effects of DAB in selecting for cells with lower ploidy can be mimicked by low doses of paclitaxel. Interestingly, the ploidy dependent mitotic arrest that is observed with DAB or low-dose paclitaxel is not detected with other chemicals that arrest cells in mitosis such as Nocodazole or the Eg5 kinesin inhibitor Monastrol, arguing that this effect is not just a consequence of increasing the time cells spend in mitosis but is specific to reagents that specifically decrease microtubule dynamics and therefore stabilize microtubule-kinetochore attachments (Figure S4D).

Based on all of the above, our model shows that while mild perturbations of the mitotic spindle might be tolerated in cells with fewer chromosomes, as all chromosomes could finally find their way to the metaphase plate, these problems become progressively acute as the chromosome number increases, leading to an irreversible mitotic arrest and cell death. In support of this concept, components of the spindle assembly checkpoint have been shown to be dispensable in organisms with few chromosomes such as flies (Buffin et al., 2007), as well as in HAP1 cells (Raaijmakers et al., 2018). Furthermore, a genome-wide screening performed in yeast revealed that mitotic spindle disturbances are preferentially essential for triploid or tetraploid cell (Storchová et al., 2006). In conclusion, in addition to its usefulness for studies using haploid cells, we should note that the capacity of DAB or low-dose paclitaxel to select for cells with lower ploidy in mixed cultures of mammalian cells might also be relevant in the context of cancer, as an average of 37% of all tumors have been found to present whole-genome duplication events in recent genomic studies (Zack et al., 2013). Given that taxanes are widely used medical compounds, we propose to focus on low doses of paclitaxel for further studies in this direction. To what extent this approach can be used as a strategy to selectively target polyploid and aneuploid cells in cancer, and whether this is actually beneficial or damaging, remains to be seen.

STAR+METHODS

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SUPPLEMENTAL INFORMATION

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AUTHOR CONTRIBUTIONS

O.F.-C. supervised the project; T.O., M.V.-S., and M.M. performed research; T.O., G.d.C., M.M., S.O., S.R., and O.F.-C. defined the experiments and analyzed the data; and T.O. and O.F.-C. wrote the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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**STAR METHODS**

**KEY RESOURCES TABLE**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Mouse Monoclonal anti-p53 | Cell Signaling | 2524; AB_331743 |
| Rabbit Polyclonal anti-CDK2 | Santa Cruz | Sc-163; AB_631215 |
| Mouse Monoclonal anti-phospho-histone H2A.X | Millipore | 05-636; AB_309864 |
| Mouse Monoclonal anti-α-Tubulin | Sigma | T9026; AB_477593 |
| Polyclonal anti-phospho-histone H3-Ser10 (pH3) | Millipore | 05-636; AB_310177 |
| anti-centromeric antibody (ACA) | Kind gift of Marcos Malumbres, CNIO, Madrid, Spain | N/A |
| **Chemicals, Peptides, and Recombinant Proteins** |        |            |
| FDA-approved compounds (977) | Selleckchem | L1300 |
| Deacetylbaccatin III | Selleckchem | S2409 |
| Paclitaxel | Sigma | T7402 |
| Nocodazole | Sigma | M1404 |
| Hoechst 33342 | Thermo Fisher Scientific | 62249 |
| Propidium Iodide (PI) | Sigma | P4170 |
| **Critical Commercial Assays** |        |            |
| Amaxa® Cell Line Optimization Nucleofector® Kit | Lonza | N/A |
| Lipofectamine 2000 (Invitrogen) | Invitrogen | 11668019 |
| CellTiter-Glo® Luminiscent Cell Viability Assay | Promega | G7570 |
| Click-iTTM EdU Alexa Fluor® | Invitrogen | C10086 |
| **Experimental Models: Cell Lines** |        |            |
| HAP1 cells | Kind gift of Thijn Brummelkamp, NKI, Amsterdam | N/A |
| DLD1 cell lines | Kind gift of Spiros Linardopoulos, ICR, London, UK | N/A |
| HEK293T | American Type Culture Collection | CRL-3216 |
| U2OS CyclinB1-164-mCherry | Kind gift of Marcos Malumbres, CNIO, Madrid, Spain | N/A |
| Mouse ESCs | Olbrich et al., 2017 | N/A |
| **Recombinant DNA** |        |            |
| SV40-T121 | Kind gift of Manuel Serrano, IRB, Barcelona, Spain | N/A |
| pX330-U6-Chimeric_BB- CBh-hSpCas9 | Cong et al., 2013 | Addgene #42230 |
| pLenti-H2B-RFP | Kind gift of Marcos Malumbres, CNIO, Madrid, Spain | N/A |
| pLVTHM | Wizinowicz and Trono, 2003 | Addgene #12247 |
| pHIV-tdTomato | Kind gift of Bryan Welm | Addgene #21374 |
| pKLV-U6gRNA(BbsI)-PGKpuro2ABFP | Addgene | Addgene #50946 |
| pVSVG | Kind gift of Oded Singer, The Salk Institute, La Jolla, USA | N/A |
| pMDLg/RRE | Dull et al., 1998 | Addgene #12251 |
| pRSV-Rev | Dull et al., 1998 | Addgene #12253 |
| **Oligonucleotides** |        |            |
| pX330-CRISPRp53-F: CACCCTGAAGCTCC CAGAATGCGAGG; pX330-CRISPRp53-R: AACACCTGACATTCT GGAGCTTCAC | Olbrich et al., 2017 | N/A |

(Continued on next page)
LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Oscar Fernandez-Capetillo (ofernandez@cnio.es).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Cell Culture
HAP1 cells (kind gift of Dr. Thijn Brummelkamp, NKI, Amsterdam) were grown in IMDM (Invitrogen) supplemented with 15% FBS (Sigma), 1% P/S and 1% Glutamine (34). Mouse embryonic fibroblasts (MEFs) were obtained from 13.5 dpc embryos using standard methods and cultured in DMEM (Invitrogen), 15% FBS and 0.1 mM non-essential amino acids in low-oxygen conditions. Feeder layers were generated from MEFs grown at early passages and growth arrested by ionizing irradiation (IR) with 80 Gy for 30 min. MEFs were immortalized by lentiviral expression of the SV40-T121 antigen following a standard protocol. Diploid and tetraploid DLD1 cell lines were a kind gift of Spiros Linardopoulos (ICR, London, UK). Cells were cultured in RPMI, 10% FBS and 1% P/S. HEK293T (American Type Culture Collection) cells and the CyclinB1-164-mCherry (Addgene #26063) expressing U2OS cell line were cultured in DMEM (Invitrogen), 10% FBS and 1% P/S. The following compounds were used to treat the cells: DAB (Deacetyl-baccatin III, S2409, Selleckchem), Paclitaxel (T7402, Sigma), Nocodazole (M1404, Sigma). A total of 5x10^5 cells were transfected with 10 mg of pX330-sgRNA-p53 plasmid (sequences provided below) using the Amaxa® Nucleofector kit (Reactive L, X-001 program) to generate P53-deficient HAP1 cells.

METHODS DETAILS

Chemical Screen
HAP1 haploid cells expressing tdTomato and HAP1 diploid cells expressing EGFP were mixed in a ratio 4:1 and seeded in 96-well plates (a total of 2500 cells per well). Cells were treated individually with a chemical library containing 977 bioactive compounds, most of which are FDA-approved (Z145127, Selleckchem) (Table S1). The screen was carried out in duplicate. Fresh compounds were added twice per week and cells passaged once per week. After 21 days of treatment, cells were trypsinized and analyzed for the expression of tdTomato and EGFP by high throughput flow cytometry (BD FACS Canto II™, BD Biosciences).

Derivation and Culture of mhaESCs
mhaESCs were generated as described recently (Leeb and Wutz, 2011) and cultured on feeder layers in gelatin-coated plates at 37°C in N2B27-based medium plus 1000 U/ml LIF, PD0325901 (1 μM), CHIR99021 (3 μM) and supplemented with 15% knockout serum replacement (Invitrogen), 0.1 mM nonessential amino acids and 0.35% BSA fraction V.

Plasmids
The plasmid pX330-U6-Chimeric_BB-CBh-hSpCas9 (Addgene, 42230) (Cong et al., 2013) was used in cells for gene editing. The sequences of the sgRNAs (pX330-CRISPRp53- F: CACCGTGAAGCTCCCAGAATGCCAG; pX330-CRISPR-p53-R: AAACCTGGCATTCTGGAGCTTCAC) used were designed and cloned as described by using the MIT CRISPR design tool (https://portals.broadinstitute.org/gpp/public/analysis-tools/sgrna-design). Only sgRNAs with the higher scores and lower probabilities of generating off-target effects were selected. pLenti-H2B-RFP was obtained from Dr. Marcos Malumbres (Cell Division and Cancer Group, CNIO, Madrid). The lentiviral plasmids pLVTHM (Addgene, 12247) (Wiznerowicz and Trono, 2003), pHIV-tdTomato (Addgene, 21374), pKLV-U6gRNA(BbsI)-PGKpuro2ABFP (Addgene, 50946) (Koike-Yusa et al., 2014) were used to express EGFP, tdTomato or BFP fluorescent proteins after infection. All de-novo generated constructs were sequenced entirely to rule out the presence of mutations.

Lentiviral Production
Lentiviral vectors were individually co-transfected in HEK293T cells using Lipofectamine 2000 (Invitrogen) with 3rd generation packaging vectors (pMDLg/RRE, Addgene #12251; pRSV-Rev, Addgene #12253 and pCMV-VSVg, kind gift of Dr. Oded Singer) to

Continued

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Software and Algorithms | | |
| GraphPad Prism | GraphPad Software Inc | http://www.graphpad.com/scientific-software/prism/ |
| Definiens Developer XD™ software | Definiens Inc. | https://www.definiens.com/ |
| Fiji softwares | Schindelin, et al., 2012 | https://imagej.net/Fiji |
| Flow Jo 10™ software | Flowjo, LLC | https://www.flowjo.com/ |

e2 Cell Reports 28, 597–604.e1–e4, July 16, 2019
generate viral supernatants (Dull et al., 1998). Lentiviral supernatants were collected 36 hours after transfection, pooled and passed through a 0.45 µM filter to eliminate cellular debris.

**Flow Cytometry**

We routinely analyzed the cell cycle profiles after cell sorting and when indicated by flow cytometry. Briefly, cells were trypsinized, stained with 10 µg/ml Hoechst 33342 for 30 minutes at 37°C and the analytic flow profiles of the DNA content was recorded on a BD Fortessa™ (BD Biosciences). At the same time or independently, we monitored and recorded the percentage of the corresponding tdTomato+, EGFP+ and BFP+ in the HAP1, DLD1 or immortalized MEF cell populations. For the evaluation of DAB toxicity, cells were fixed in suspension with 70% ethanol and stained with PI (100 µg/ml) for DNA content analyses.

**Flow Activated Cell Sorting (FACs)**

HAP1, immortalized MEFs and mhaESCs were trypsinized followed by Hoechst staining (10 µg/ml Hoechst 33342, Thermo Fisher Scientific) for 30 minutes at 37°C. Sorting for specific haploid, diploid or tetraploid cell populations was based on DNA content and/or expression of tdTomato, EGFP or BFP on a BD Influx™ cell sorter (BD Biosciences). In a mix culture of haploid/diploid cells, haploids were sorted based on the G1 haploid (1n) and diploids on the G2/M diploid (4n) peaks, respectively. In a mix culture of diploid/tetraploid cells, diploids were sorted based on the G1 diploid (2n) and G2/M tetraploid (8n) peak, respectively. After each sort the purity of the sorted cells was carefully checked by flow cytometry analysis.

**Western Blot**

HAP1 cell pellets were lysed in 50mM Tris, 150mM NaCl, 1% Triton X-100 or in 50mM Tris pH 7.9/8M Urea/1%Chaps followed by 30 min of incubation time shaking at 4°C. 15-25 µg of supernatants were run on precast gels and transferred for protein detection by using the following antibodies: p53 (1:1000, Cell Signaling, #2524); CDK2 (1:2000; sc-163, Santa Cruz).

**Immunofluorescence**

Cells were fixed with 4% PFA followed by permeabilization with 0.1% Triton X-100. Antibodies against γH2AX (1:1000, Millipore 05-636), αTubulin (1:1000, Sigma #T9026), phospho-histone H3-Ser10 (pH3) (1:100, Millipore 06-570), anti-centromeric antibody (ACA, 1:500, obtained from Marco Malumbres, Cell Division and Cancer Group, CNIO, Madrid) were used. Images were acquired using a Leica TCS-SP5 equipped with a 0.7 NA 20x oil or 1.4 NA 63x oil (HCX plan Apo CS) objective and LAS AF 2.6 software.

**High-Throughput Microscopy (HTM)**

High-throughput microscopy analyses were carried out as described in (Lopez-Contreras et al., 2013). 10000 HAP1 cells were seeded per well on μClear® bottom 96-well plates (Greiner Bio-One). Immunofluorescence staining with the indicated antibodies (γH2AX or phospho-H3) was performed (see above) and images from each well were automatically acquired using an Opera High-Content Screening System (Perkin Elmer). The images were taken at non-saturating settings and segmented using the DAPI staining to generate masks matching cell nuclei. The levels of EdU incorporation was measured by adding EdU to the media at a final concentration of 20 µM for 30 minutes. For EdU detection the Click-iT™ EdU Alexa Fluor® imaging kit (Invitrogen™) was used.

**Metaphase Spreads**

HAP1 cells were arrested at mitosis with overnight treatment with 100 ng/ml Colcemide (GIBCO/BRL). Cells were then collected, incubated in a hypotonic buffer (0.075 mM KCl) for 15 min at 37°C and fixed with Carnoy’s buffer (methanol-glacial acetic acid, 3:1). To obtain metaphase spreads, cells were dropped on slides and stained with Giemsa solution. Images from metaphases were captured and a minimum of 19 metaphases were analyzed.

**Live Cell Imaging**

To evaluate mitosis entry and duration, HAP1 cells were infected with lentiviruses encoding the histone H2B-RFP and seeded on 8 wells μ-Slide (Ibidi, 80826). The day after, the cells were treated with the indicated compounds and imaged every 4 minutes for a total of 16h with a 20x objective in a Leica DMI 6000 B system. For a more careful evaluation of mitosis, SAC-dependent time was defined as the time from nuclear envelope breakdown (NEBD) until the observation of a metaphase plate and SAC-independent time from metaphase plate to chromosome decondensation. At least, 35 cells were followed to evaluate the time spent in mitosis and interphase as well as cell fate for each individual cell. To evaluate cyclin B degradation, U2OS cells expressing a CyclinB-mCherry fusion protein were seeded on 8 wells μ-Slide (Ibidi, 80826). The following day, cells were treated with the indicated compounds and imaged every 4 minutes for a total of 16h in a Leica DMI 6000 B system. Cyclin B degradation was evaluated in cells from the NEBD, with the maximum level of cyclin B expression, until the start of anaphase, where mCherry signal was lost entirely.
Cold Shock Treatment
U2OS cells were pretreated with 10 μM DAB for 1 hour at 37°C. Then, cells were placed on ice and put in ice-cold media supplemented with HEPES (pH 7.4) for 30 min to allow microtubule depolymerization. Finally, cells were either fixed with 4% PFA or placed back to regular media with or without 10 μM DAB at 37°C and fixed after 3 min. Immunofluorescence staining was performed as described above.

QUANTIFICATION AND STATISTICAL ANALYSIS
Where indicated, data are represented as means ± standard error of the mean. Differences between groups were evaluated by two-way analysis of variance (ANOVA) and P values are indicated when differences between two groups were statistically significant (< 0.05). Statistical analyses were performed using Prism 8 (GraphPad La Jolla, CA).
Supplemental Information

A Chemical Screen Identifies Compounds Capable of Selecting for Haploidy in Mammalian Cells

Teresa Olbrich, Maria Vega-Sendino, Matilde Murga, Guillermo de Carcer, Marcos Malumbres, Sagrario Ortega, Sergio Ruiz, and Oscar Fernandez-Capetillo
**Figure S1.** (related to *Fig 1*) Flow cytometry analysis of DNA content in HAP1 cells that were sorted for haploidy by FACS on day 0 and after 26 days of growth in the presence of DMSO (control), Regorafenib (10 μM) or Nilotinib (10 μM). The fraction of haploid G1 cells is highlighted in red. These compounds provide a modest stabilization of haploidy in HAP1 cells (the effect of DAB is shown for comparison), although they also have a general effect in slowing down the growth of these cells, which simply slows down the overgrowth of haploid HAP1 by diploid cells. Note that the control and DAB are the same as the ones provided in Fig. 1C, as these data belong to the same validation experiment.
Figure S2. (related to Fig 2) High-Throughput Microscopy (HTM) analysis of the phosphorylation of histone H2AX (γH2AX) levels (left panel) or DNA replication rates as measured by the levels of EdU incorporation per nucleus (right panel), in haploid HAP1 cells exposed to the indicated doses of DAB for 24 hrs. Doxorubicin (10 μM, 30 min) was used as a positive control for a DNA damaging agent. A 30 min pulse of EdU was given after the 24 hr period. A dashed line indicates mean values. One representative example out of two independent experiments is shown.
Figure S3. (related to Fig 4) (A) Flow cytometry analysis of DNA content in wild type (WT) and P53-deficient (p53KO) HAP1 cells that were sorted for haploidy by FACS on day 0 and after 25 days of growth in the presence of DMSO (control) or DAB (10 μM). The fraction of haploid G1 cells is highlighted in red. One representative example out of two independent experiments is shown. On the right of the panel, a western blot illustrating the absence of P53 expression in the P53-deficient HAP1 clone used for these analyses. The clone was generated by CRISPR/Cas9 through infection with lentivirus expressing a P53-targeting sgRNA. (B) The effect of DAB (10 μM) in microtubule dynamics was assessed in U2OS by inducing microtubule depolymerization with a cold shock (placing cells in ice-cold media supplemented with Hepes (pH 7.4) for 30 min) and staining for alpha-TUBULIN (green) at time 0, or 3 min after placing the cells back to 37°C. The compound had a clear impact on the reorganization of microtubules within the cell, evidenced by an accumulation of alpha TUBULIN at centrosomes and aggregates throughout the cell. Scale bar (white) indicates 2.5 mm. DAPI was used to stain DNA.
Figure S4. (related to Fig 4) (A) Flow cytometry analysis of DNA content in haploid, diploid and tetraploid HAP1 cells grown in the presence of DMSO (control) or DAB (10 or 20 μM) for 24 hrs, illustrating the ploidy-dependent toxicity of this compound in HAP1 cells. (B) Effect of DAB (10μM) on the growth of haploid, diploid and tetraploid HAP1 cells. One representative example out of two independent experiments is shown. Data are represented as mean ± SEM, ***p<0.001. (C) Immunofluorescence of alpha TUBULIN (αTUBULIN, blue), phosphorylated histone H3 (H3S10, red), and the centromeric marker ACA (green) in haploid, diploid and tetraploid HAP1 cells treated with DAB (10 μM) for 16 hrs. While normal metaphase and anaphase figures can be found in haploid cells (yellow arrowheads), mitotic figures of diploid and most prominently tetraploid cells present evidences of lagging chromosomes or a complete failure to assemble a metaphase plate. Scale bar (white) represents 2.5 μm. (D) Evaluation of the mitotic arrest induced after a 16 hr treatment with DAB, Nocodazole or Monastrol at the indicated doses in haploid (n), diploid (2n) and tetraploid (4n) HAP1 cells. The mitotic index was calculated by High-Throughput Microscopy through the quantification of cells presenting phosphorylation of histone H3 at Ser 10. Data are represented as mean ± SEM.