Supplementary Table Legends

Supplementary Table S1: Chromosome segregation error rates per chromosome used to determine average missegregation rates in our studies.

The error rate per chromosome is indicated for the results presented in Fig. 1A&F and Supp. Fig. 3F. For each experiment, we used Fisher's exact test to determine whether the error rate was significantly different between chromosomes.

Supplementary Table S2: Gene ontology enrichment analysis from whole-genome RNAi screen identifies APC/C subunits.

Gene ontology enrichment analysis was performed using DAVID 6.8 on the top ~700 genes, identifying APC/C subunits within the most significantly enriched categories.

Supplementary Table S3: 30 TCGA studies used in this paper.

List of the 30 tumor types used to determine the overall mutation frequency (non-synonymous mutations) involving an APC/C gene subunit. The cancer type and number of samples for which sequencing data was available is indicated (data extracted from cBioPortal). Only studies from The Cancer Genome Atlas were selected to maximize consistency across sequencing platforms. TCGA accession number: phs000178.v9.p8

Supplementary Table S4: APC/C mutation frequencies in TCGA.

TCGA data as compiled on cBioPortal for the 30 cancer types listed in Table S3, indicating the number of mutations identified for each subunit, and the fraction of those mutations that are truncating mutations (nonsense, frameshift or splice site mutations).

Supplementary Table S5: Structural insight into the impact of cancer-associated APC/C mutations.

Details regarding the predicted impact of APC/C missense mutations based on the APC/C atomic structure.

Impact on the structural integrity of APC/C was based on the published APC/C structure (1) which contains Cdh1 as a co-activator instead of CDC20. The functional consequence of CDC20 mutations may be hard to interpret since CDC20 is also part of the mitotic checkpoint complex (MCC), which is responsible for delaying anaphase onset. For example, R132P, which is located in the KILR motif, could disrupt MCC formation and the co-activator's interaction with the APC/C (2), and result in impaired MCC formation in cells similar to what previously reported with the R132A mutation (3). Mutation Assessor and Polyphen-2 were also used to identify potentially deleterious mutations. *S. pombe* and human Cdc27 protein sequences were aligned using EMBOSS Needle, and the BLOSUM62 score was used to indicate the degree of conservation for the residues of interest.

Supplementary Figure Legends

Supplementary Figure S1: Using reversine to cause chromosome segregation errors and p53-dependent aneuploidy tolerance.

A. Immunostaining of p53 and p21 48h following transfection with TP53 or control siRNA in 500nM reversine.

B. Automated image acquisition and quantification three days post-siRNA transfection to determine cell number, the percentage of cells expressing p21, cyclin A (by immunostaining - S/G2 marker) or cells that have incorporated the nucleoside analog 5-ethynyl-2'-deoxyuridine (EdU - therefore in S phase) which was added to the media 1h prior to fixation. Fold differences for each parameter are shown in blue, revealing that TP53 RNAi results in a higher proliferative index than control siRNA in reversine, indicative of aneuploidy tolerance.

C. HCT116 cells were treated for 48h with reversine, fixed and stained for tubulin, MKLP1 (to confirm anaphase onset) and DAPI. The severity of segregation errors was classified empirically based on the severity of segregation errors to highlight that higher reversine concentrations more often cause severe anaphase defects (light blue being most severe).

D. HCT116 wt and p53-/- cells were grown in reversine for the indicated time and cell lysates were analyzed by SDS-PAGE and western blot.

E. Sample images (automated acquisition/analysis) showing increased EdU incorporation 48h after TP53 RNAi in HCT116 cells treated with 250nM reversine.

F. A single passage through mitosis in the presence of reversine is sufficient to cause cell cycle arrest/death in the following interphase.

Single cell tracking was set up to follow cell fate after a single division in reversine or following division that occurred after reversine washout (hence when the mother cells was only exposed to reversine during interphase, not in mitosis). As shown, reversine exposure during interphase

does not cause cell cycle arrest. Cell cycle arrest following a division in reversine quantified here all occurred in the first interphase and did not require a second passage through mitosis.

G. Reversine treatment does not result in p53 phosphorylation at Serine 15, an ATM phosphorylation site. Cells were maintained in 250nM reversine for 48h or 1μM doxorubicin for 24 (as a positive control), fixed and immunostained using phospho-specific antibodies against pS15-p53.

H. The ATM inhibitor KU-55933 (ATMi - at 5-10-30μM) prevents p21 expression following DNA damage induction with doxorubicin but not after induction of segregation errors with reversine, determined by quantifying the fraction of p21-expressing cells by immunofluorescence.

Supplementary Figure S2: Validation of APC/C subunits using siRNA deconvolution experiments in RPE1 and HCT116 cells, and how subunit knock-down rescues proliferation when the spindle assembly checkpoint is impaired.

A. Stills from time-lapse movies (IncuCyte) 72h after siRNA transfection, showing ongoing proliferation in 500nM reversine upon APC/C RNAi. Note that without reversine more cells accumulate in mitosis (round cell), as expected if APC/C function is compromised.

B. Table summarizing the results of deconvolution experiments, indicating the number of single siRNA oligos that increased EdU incorporation when cells are grown in 250nM reversine, from the siGenome and On-Target-PLUS (OTP) Dharmacon commercial collections.

C. Example of a small-scale deconvolution experiment showing that most TP53, CDC16, ANAPC10 and UBE2C siRNA oligos increase cell number and EdU incorporation in 250nM reversine.

D. Deconvolution experiment of the CDC27 OTP pool, along with TP53 oligos. Western blot showing CDC27 knock-down was performed with cell lysates collected in parallel.

E. siRNA transfection of various siRNA pools against APC/C subunits in HCT116 cells in the presence of 200nM reversine. The effect on cell cycle proliferation was quantified by EdU incorporation/detection (1h EdU labeling). Six non-targeting control siRNAs were used (white bars). Cells were fixed 4 days following siRNA transfection.

F. CDC27 deconvolution experiment in HCT116 cells using the same CDC27 OTP oligos as in (D). Cells were transfected with each siRNA in the presence of 200nM reversine. Here cells were fixed 3 days post-transfection, explaining the higher fraction of EdU incorporation compared to Fig. S2E.

Supplementary Figure S3: APC/C subunit knock-down reduces segregation errors caused by SAC defects.

A. RPE1 cells were transfected with different combinations of OTP siRNAs against MAD2 (used here instead of reversine to impair the SAC) together with CDC27 or TP53 siRNAs, as indicated. Cells were labeled with EdU prior to fixation, and automated acquisition/analysis was used to quantify the fraction of S phase cells (EdU+) and estimate the number of micronuclei normalized to total cell number.

B. Co-transfection of siRNA against Mad2 together with ANAPC10 or TP53 siRNA pools (Dharmacon siGenome) in RPE1 cells.

C. HCT116 cells were treated and analyzed as in (A) (left). Sample images of DAPI stained nuclei are shown, following Mad2 and CDC27 siRNA transfection, showing that co-depletion restores normal nuclear morphology.

D. Experimental procedure for determining chromosome segregation error rates after CDC16 siRNA or transient MG132 treatment (10μM). 48h post-transfection, cells were synchronized at the G1/S transition with a single thymidine block. Thymidine was then washed off to allow cell cycle progression and reversine was added 10h after thymidine release (just prior to entry to

mitosis). Mitotic cells were collected at 12h after thymidine release by gentle shake-off and allowed to re-attach on coverslips in the presence of reversine and fixed. Chromosome segregation error rates were determined by FISH in newly born daughter cells using the indicated centromeric probes, showing 13.9-fold reduction with CDC16 RNAi and 24.7-fold decrease with MG132.

E. Western blot analysis of CDC16 levels from lysates obtained at time of fixation.

F. Cells were treated for 1h with 10μM MG132 before shake-off to arrest cells at metaphase. MG132 was washed off to allow cells to divide and cells were fixed 2h later as soon as they re-attached on glass coverslips (where 500nM reversine was added, it was added 10h after thymidine release until cell re-attached, and also kept during MG132 treatment).

G. Mitotic duration (NEBD-anaphase) following APC10 or CDC16 siRNA transfection with or without 500nM reversine determine by phase-contrast microscopy (IncuCyte) every 3 minutes.

Supplementary Figure S4: CRISPR-mediated disruption of TP53 and CDC27, and resistance to Mps1 inhibitors.

A. Western blot analysis of steady-state CDC27 levels in CDC27-CRISPR populations after selection in reversine. Note that the Vector control comes from cells that did not go through reversine selection and were acutely treated with 250nM reversine. Note that p53 levels are similar, indicating that CDC27 disruption does not impair p53 stabilization.

B. Sanger sequencing data to detect CDC27 disruption at each CRISPR/Cas9 site. The number of disrupted clones is indicated, along with clones isolated over the same period but without reversine selection, to measure the background disruption rate.

Supplementary Figure S5: Functional testing of CDC27 mutations in Schizosaccharomyces pombe.

A. Diagram showing the mating schema and possible genotypes of haploid spores, as performed to test the functionality of CDC27 (Nuc2) truncating and missense mutations. *S. pombe* and human Cdc27 protein sequences were aligned using EMBOSS Needle, and the BLOSUM62 score was used to indicate the degree of conservation for the residues of interest. Seven missense mutations with a BLOSUM62 score above 2 were tested in yeast (homologous to human V25F, L144V, P246S, R266Q, G506E, D597H and R629K, see Supplementary Table S5).

B. Cyclin-GFP degradation was measured during mitosis for Nuc2 deleted haploid cells complemented either with a wild-type Nuc2 gene (n=169) or the R493K mutant (n=172), which corresponds to R629K in human. Decay curves are normalised to peak levels of cyclin-GFP.

C. Atomic structure of the human CDC27 homodimer depicting the interaction with the IR-tail of the co-activator and the IR tail of APC10 during substrate recognition (1,4). ProteinDatabaseBank accession number 4UI9. Please refer to (4) for a detailed analysis.

D. Phase-contrast images of RPE1 populations expressing the indicated lentiCRISPR vectors following continuous treatment in 250nM reversine for 30 days following viral infection. An empty lentiCRISPR vector as well as a vector expressing H2B-mCherry were used as controls. Note the morphology of the rare control cells that survive in contrast with the highly proliferative populations resulting from APC/C subunit disruption. Lollipop plots indicating the location of truncating mutations reported by TCGA (extracted from cBioportal) along with the location of the protospacer sites used to design CRISPR/Cas9 guide RNAs.

Supplementary Figure S6: Cell division, microtubule attachment errors and tetraploidy.

A. (i) During prometaphase the SAC is activated due to unattached kinetochores (eg. monotelic) and the lack of tension between kinetochores (syntelic). The mitotic checkpoint complex

prevents full APC/C activation by sequestering CDC20, but some APC/C targets such as cyclin A2 and Nek2 can be degraded with an active checkpoint. Merotelic attachments, where one of the two kinetochores is attached to both poles, do not trigger SAC engagement and therefore rely on error correction mechanisms to be resolved.

(ii) Upon chromosome alignment at metaphase, the checkpoint is 'satisfied', resulting in APC/C^{CDC20} full activation and mitotic progression.

(iii) Unresolved merotelic attachment can lead to lagging chromosomes during anaphase, whose random segregation can lead to aneuploid progeny.

(iv) Lagging chromosomes might not reach the main chromosome mass before nuclear envelope re-formation, and thus become isolated into micronuclei.

(v) Merotelic attachments can be generated experimentally by preventing centrosome separation in early mitosis (eg. STLC or monastrol) which causes mitotic arrest. Upon drug release bi-orientation is achieved and cells progress to anaphase.

B. Genome-doubling (for example through cytokinesis failure) causes cells to enter the following mitosis with twice as many centrosomes, creating a transient multipolar spindle, increasing the chance of creating merotelic attachments. Multipolar configurations can satisfy the SAC leading to multipolar divisions, most of which leading to catastrophic karyotype changes.

Supplementary Figure S7: CRISPR-mediated disruption of TP53 in RPE1 cells.

A. Two populations of RPE1/p53null cells were generated with lentiCRISPR using the indicated guide RNAs targeting adjacent sites within the first exon, in order to control for potential off-target cleavage.

B. Cells were infected with lentiCRISPR vectors at low titre and selected with 5µg/ml puromycin for two weeks in culture. Post-selection, whole populations were UV-irradiated to induce p53 and assess TP53 status. Both TP53 CRISPR populations showed very low levels of p53 by western

blot, similar to basal levels in control RPE1 cells, allowing us to conclude that the two polyclonal cell lines are essentially p53-null. Population 1 was used for the experiments in Figures 3 and 5.

Supplementary Figure S8: APC/C partial inhibition does not rescue structural anaphase defects caused by pre-mitotic replicative stress.

A. Effect of proTAME on NEBD-anaphase in HCT116 cells. Time-lapse movies were taken (every three minutes) 2h after addition of proTAME. No cell was delayed in mitosis for more than 80 minutes, indicating that APC/C was only partially impaired (Bars = average with 95% CI). In addition, all cells achieved anaphase and cytokinesis.

B. HCT116 cells were treated for 18h with 200nM aphidicolin (APH) to induce replication stress, released in fresh media for 4h and fixed in methanol. Where indicated, proTAME was added 2h after APH washout and cells were fixed 2h later (4h total). Cells were analyzed by immunofluorescence following staining using anti-tubulin and anti-centromere (CREST) antibodies along with DAPI. As shown, proTAME does not reduce the frequency of structural aberrations in mitosis caused by replication stress. Note that proTAME concentrations used in all our experiments are well below those causing robust APC/C inhibition, mitotic arrest and eventually mitotic slippage (5μM to 12μM depending on the cell line (5)).

Supplementary Figure S9: Spindle microtubule stability using photoactivation.

A-B. We generated RPE1 cells stably expressing mCherry-photoactivatableGFP-Tubulin, in order to determine whether proTAME treatment destabilizes spindle microtubules when cells progress through prometaphase. To do this, two sequential photoactivations were performed in each cell five minutes appart. The difference in tubulin half-life between the first and second photoactivations was determined for cells treated with DMSO or 3μM proTAME. The mitotic spindle was visualized as it formed in early mitosis using the mCherry channel, and GFP

photoactivation was done in a narrow region off-centered near the middle of the cell. Following photoactivation, the decaying intensity of the GFP signal was quantified within the photoactivated region and used to determine the tubulin half-life (see experimental procedures).

Supplementary Figure S10: Adaptation of RPE1/p53ko populations to Mps1 inhibitors is accompanied by a mitotic delay.

A. Doubling time of control and p53-disrupted RPE1 populations while continuously maintained in 250nM reversine for up to 44 days.

B. RPE1/lentiCRISPR vector, p53ko-1 or p53ko-2 populations (generated with guide RNAs shown in Supplementary Fig. S7A) were maintained continuously in 2μM AZ3146 for 51 days. AZ3146 is an Mps1 inhibitor structurally unrelated to reversine. Both p53-null populations were proliferating after 51 days in AZ3146, as opposed to RPE1/lentiCRISPR/vector cells. AZ3146 removal caused a delay in mitotic progression indicated here by an apparent accumulation of mitotic cells.

Supplementary references

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