**LEGENDS TO SUPPLEMENTARY FIGURES AND VIDEOS**

**Figure S1. The absence of p53 is permissive for polyploidization.** A-C. Wild type (WT) and p53^+/− tetraploid HCT 116 cells were treated with 100 nM nocodazole (Noco) or 1 cytochalasin D (Cyt D) for 48 h and then processed for the quantification of ploidy and apoptosis-associated parameters. This was achieved by cytofluorometric analysis upon three-color staining with Hoechst 33342 (which measures DNA content), the mitochondrial transmembrane potential (Δψ_m)-sensitive dye 3,3′-dihexyloxacarbocyanine iodide (DiOC₆(3)) and propidium iodide (PI, an exclusion dye that is incorporated only into dead cells). Representative FACS pictograms are shown in panel A (the percentage of cells found in relevant quadrants is reported), while quantitative results are presented in panels B and C (mean ± SEM, n = 5 independent experiments). In B, columns depict the percentage of polyploid cells quantified among total cell populations. Black and white columns in C report the percentage of dead (characterized by plasma membrane permeabilization, PI/Δψ_m^low) and dying (which exhibit Δψ_m dissipation but intact plasma membranes, PI/Δψ_m^low) cells, respectively. Asterisks indicate significant differences (Student’s *t*-test, *p*<0.05) between p53^+/− and WT cells subjected to the same treatment.

**Figure S2. Inhibition or depletion of p53 stimulates the generation of sub-tetraploid cells from tetraploid precursors.** A. Pharmacological inhibition of p53. Freshly generated p53-proficient tetraploid RKO cells were FACS-purified after Hoechst 33342 staining (as described for HCT 116 cells in Figure 1A) and cultured in the absence or in the presence of cyclic pifithrin-α, a pharmacological inhibitor of p53. After the indicated number of days, cells were stained with propidium iodide (PI) for the cytofluorometric quantification of DNA content. The histogram represents the percentage of sub-tetraploid cells generated by p53 inhibition (mean ± SEM, n = 3 independent experiments). Asterisks highlight significant differences between the percentage of sub-tetraploid cells induced by pifithrin-α and those observed in untreated cells (Student’s *t*-test, *p*<0.05). B. Prolonged depletion of p53 causes aneuploidization of wild type tetraploid HCT 116
cells. Wild type (WT) tetraploid HCT 116 cells were repeatedly transfected with a control (UNR) or a p53-specific (p53_2) siRNA approximately every 5 days. Seventy-two hours after the indicated transfection, cells were subjected to cytofluorometric DNA content analysis upon propidium iodide staining. In 10 out of 12 experiments, prolonged depletion of p53 led to the generation of a “phase 1” profile, characterized by a rather broad shoulder of sub-tetraploid cells. In the other 2 instances, a “phase 2” profile (with a sharp peak of sub-tetraploid cells) was observed, as indicated.

**Figure S3.** Comparative assessment of the proliferation of phase 1 versus phase 2 unstable tetraploid p53⁻/⁻ cells. Unstable tetraploid p53⁻/⁻ HCT 116 cells of the indicated phase were stained with carboxyfluorescein diacetate succinimidyl ester (CFSE) and fluorescence intensity (which diminishes with each cell division) was determined every 24 h on 4 consecutive days (A), or the number of cells was cytofluorometrically determined with the help of counting beads (mean ± SEM, n = 3) (B). Alternatively, phase 2 tetraploid p53⁻/⁻ HCT 116 cells were stained with Hoechst 33342, subjected to the cytofluorometric sorting of sub-tetraploid (near-to diploid, ~2n) and tetraploid (>4n) population, and then stained with CFSE to assess proliferation as in A (C). Moreover, phase 2 tetraploid p53⁻/⁻ HCT 116 cells expressing a histone 2B-green fluorescent protein (H2B-GFP) chimera were sorted into near-to-diploid and tetraploid cells as in C, followed by the monitoring of these sub-populations by videomicroscopy to determine the length of mitoses (mean ± SEM, n = 400 cells) (D).

**Figure S4.** Single versus repeated reversion from tetraploidy. p53⁻/⁻ HCT 116 cells were exposed to 100 nM nocodazole for 48 h, followed by Hoechst 33342 staining and FACS purification (2-4 days after nocodazole discontinuation) of tetraploid cells (DNA content >4n). Grey columns depict the % of sub-tetraploid cells arising from such 1st line tetraploid populations over time (mean ± SEM, n = 4). Alternatively, near-to-diploid clones that originated from 1st line tetraploid cells were re-tetraploidized by means of the same protocol, and the frequency of the reversion to sub-tetraploidy was assessed. Black columns illustrate the % of sub-tetraploid cells arising from such 2nd line...
tetraploid populations over time (mean ± SEM, n = 4).

**Figure S5.** Fate of diploid cells or p53<sup>-/-</sup> sub-tetraploid clones. p53<sup>-/-</sup> diploid cells and tetraploid HCT 116 clones expressing a histone 2B-green fluorescent protein chimera (H2B-GFP) were subjected to FACS-purification of diploid or sub-tetraploid populations. Purified cells with a ~2n DNA content were then monitored by fluorescence videomicroscopy for 40 h. Only cells entering mitosis within the first 10 h were tracked until the end of the experiments. Here, all the observed cell fates are represented and their % is reported (mean ± SEM, n = 3 independent experiments, 100-150 cells analyzed). Note that cytokinesis failure (leading to an increase in ploidy as well as in cell volume) is depicted with a duplication in bar thickness. A excerpt of this figure that depicts only the most frequent cell fates can be found in Figure 4F.

**Figure S6.** Influence of Mos on the generation of sub-tetraploid cells. A. Effect of Mos transfection on the fate of tetraploid cells. Stable p53<sup>-/-</sup> HCT 116 cells were transfected for 48 h with a polycystronic plasmid for the expression of green fluorescent protein (GFP) alone or together with Mos. GFP<sup>+</sup> cell populations were FACS-purified, cultured for 48 h, and then subjected to subsequent rounds of transfection with the same constructs at intervals of ~5 days. The percentage of sub-tetraploid cells recorded after three rounds of transfection is indicated. B. Effect of Mos depletion on tetraploid cells. Phase I unstable p53<sup>-/-</sup> HCT 116 cells were transfected with a control (UNR) or a Mos-depleting siRNA for 72 h followed by DNA content analysis. The percentage of sub-tetraploid cells is indicated. Both panels A and B illustrate single experiments that are representative for the quantitative data provided in Figure 6B and 6C, respectively, where the results of three independent experiments are reported (mean ± SEM).

**Figure S7.** Mos depletion does not affect cellular proliferation. Stable wild type (WT) and unstable p53<sup>-/-</sup> tetraploid HCT 116 clones were labeled with 5-(and-6)-carboxyfluorescein diacetate succinimidyl ester (CFSE) for 24 h and then transfected with a control (UNR) or Mos-specific (Mos_1 and Mos-2) siRNAs, followed by daily assessment of cell proliferation by cytofluorometry.
Day 0 = transfection day. Fluorescence histograms are representative of three independent experiments with similar results.

**Figure S8.** Effects of Mos on centrosome coalescence in p53−/− tetraploid clones. Single-channel microphotographs of the immunofluorescence pictures shown in Figures 7A (A) and 7C (B). c: centrosome; ca: active centrosome; ci: non-active centrosome;

**Figure S9.** Knockdown of Mos-GFP by a siRNA specific for Mos. An unstable p53−/− tetraploid HCT 116 clone was transfected with a control (UNR) or a Mos-specific (Mos_1) siRNA for 24 h, followed by further transfection with a plasmid encoding green fluorescent protein (GFP) or with a construct for the expression of a Mos-GFP chimera. Two days later, cells were stained with the vital dye propidium iodide (PI, which is incorporated only into dead cells) and subjected to cytofluorometric quantification of GFP expression. Percentages refer to the fraction of living (PI−) GFP+ cells. This experiments has been repeated twice, yielding similar results.

**Figure S10.** Efficacy of siRNA-mediated depletion of HSET. Stable wild type and phase 1 unstable p53−/− tetraploid HCT 116 cells were transfected with a control siRNA (UNR) or with a siRNA specific for HSET (HSET_1) for 48 h, followed by immunoblotting with antibodies that recognize HSET and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, monitored as a loading control).

**Video S1.** Example of tripolar mitosis. Isogenic WT and p53−/− diploid HCT 116 cells expressing a histone 2B-green fluorescent protein (H2B-GFP) chimera were treated for 48 h with nocodazole and then cultured in drug-free medium for additional 48 h. Polyploid cells were FACS-sorted as in Figure 1A (>4n, black symbols) and monitored by fluorescence videomicroscopy for 48 h. This video shows a successful tripolar mitosis of a p53−/− tetraploid HCT 116 cell, which exhibits the representative Y-shaped metaphase and proceeds until completion, thereby generating three daughter cells. Representative snapshots can be found in Figure 2A.
**Video S2.** Example of tetrapolar mitosis. Isogenic WT and *p53<sup>-/-</sup>* diploid HCT 116 cells expressing a histone 2B-green fluorescent protein (H2B-GFP) chimera were treated for 48 h with nocodazole and then cultured in drug-free medium for additional 48 h. Polyploid cells were FACS-sorted as in Figure 1A (>4n, black symbols) and monitored by fluorescence videomicroscopy for 48 h. This video shows a successful tetrapolar mitosis of a *p53<sup>-/-</sup>* tetraploid HCT 116 cell, which exhibits the representative X-shaped metaphase and proceeds until completion, thereby generating four daughter cells. Representative snapshots can be found in Figure 2A.

**Video S3.** Fate of cells generated from *p53<sup>-/-</sup>* tetraploid HCT 116 clones by multipolar mitosis. *p53<sup>-/-</sup>* diploid HCT 116 cells expressing a histone 2B-green fluorescent protein (H2B-GFP) chimera were treated for 48 h with nocodazole and then cultured in drug-free medium for additional 48 h. Polyploid cells were FACS-sorted as in Figure 1A (>4n, black symbols) and daughter cells originating from this population by multipolar mitosis were monitored by fluorescence videomicroscopy for 48 h. This video shows a successful tripolar mitosis leading to the generation of three daughter cells, of which one enters and terminates a normal bipolar division, while the two others die. Representative snapshots can be found in Figure 2C.

**Video S4.** Fate of sub-tetraploid cells derived from *p53<sup>-/-</sup>* tetraploid HCT 116 clones. Sub-tetraploid cells derived from phase 1 and 2 unstable *p53<sup>-/-</sup>* tetraploid HCT 116 clones or the 2n population of the parental *p53<sup>-/-</sup>* diploid cell line were FACS-purified as described in Figure 4A, and monitored by videomicroscopy for the following 40 h. This video shows one sub-tetraploid HCT 116 cell derived from a phase 2 unstable *p53<sup>-/-</sup>* clone that undergoes two consecutive rounds of mitosis. Representative snapshots can be found in Figure 4E (example 1).

**Video S5.** Fate of sub-tetraploid cells derived from *p53<sup>-/-</sup>* tetraploid HCT 116 clones. Sub-tetraploid cells derived from phase 1 and 2 unstable *p53<sup>-/-</sup>* tetraploid HCT 116 clones or the 2n population of the parental *p53<sup>-/-</sup>* diploid cell line were FACS-purified as described in Figure 4A, and monitored by videomicroscopy for the following 40 h. This video shows one sub-tetraploid HCT 116 cell
derived from a phase 2 unstable $p53^{-/-}$ clone that generates two daughter cells in turn undergoing apoptosis. Representative snapshots can be found in Figure 4E (example 2).

**Video S6.** Fate of sub-tetraploid cells derived from $p53^{-/-}$ tetraploid HCT 116 clones. Sub-tetraploid cells derived from phase 1 and 2 unstable $p53^{-/-}$ tetraploid HCT 116 clones or the 2n population of the parental $p53^{-/-}$ diploid cell line were FACS-purified as described in Figure 4A, and monitored by videomicroscopy for the following 40 h. This video shows one sub-tetraploid HCT 116 cell derived from a phase 2 unstable $p53^{-/-}$ clone that undergoes mitosis followed by incomplete cytokinesis, resulting in increased cell volume and ploidy. A successful division was also shown. Representative snapshots can be found in Figure 4E (example 3).

**Video S7.** Effects of Mos on centrosome and chromosome dynamic in $p53^{-/-}$ tetraploid HCT 116 clones. A phase 2 unstable $p53^{-/-}$ tetraploid HCT 116 clone expressing a histone 2B-green fluorescent protein (H2B-GFP) chimera was transfected with a control (UNR) together with a centrin-DsRed fusion-encoding construct. Then mitoses were monitored by videomicroscopy. Video shows a successful tripolar division. Representative snapshots can be found in Figure 7E.

**Video S8.** Effects of Mos on centrosome and chromosome dynamic in $p53^{-/-}$ tetraploid HCT 116 clones. A phase 2 unstable $p53^{-/-}$ tetraploid HCT 116 clone expressing a histone 2B-green fluorescent protein (H2B-GFP) chimera was transfected with a Mos-specific siRNA together with a centrin-DsRed fusion-encoding construct. Then, mitoses were monitored by videomicroscopy. Video shows a bipolar divisions exhibiting centrosome coalescence in two clusters. Representative snapshots can be found in Figure 7E.
Figure S1
Figure S3