Supplementary Figure Legends

Figure S1 Gradual degradation of cyclin B1 during taxol-induced mitotic arrest.

A-D U2OS cells were synchronized by thymidine block and released into medium containing taxol. At 15 h after thymidine release, mitotic cells were collected by shake-off, further cultured in the taxol medium for the indicated times in the absence (A, B) or presence (C, D) of 50 μM cycloheximide (CHX), stained with MPM2 or anti-cyclin B1 antibodies and PI, and subjected to FACS analysis. The FACS graphs of the indicated samples are shown in (A) and (C). The normalized counts of cells with 4C DNA content (as a percentage of the maximum in the histogram of each timepoint) were plotted against the intensities of MPM2 (top panels) or cyclin B1 (bottom panels) in log scales in (B) and (D). The MPM2 and cyclin B1 intensities of mitotic cells and the adapted cells are indicated by black and red arrows.

Figure S2 $p31^{comet}$ depletion accelerates mitotic apoptosis.

A RPE1 cells transfected with the indicated siRNAs, treated with taxol and monitored by time lapse microscopy. The cumulative percentage of cells in mitosis is plotted against the time in mitosis. t_{50}(siLuc+siLuc) = 4.5 h, t_{50}(siLuc+siP31-1) = 7.4 h, t_{50}(siLuc+siCdc20) = 13.6 h, t_{50}(siLuc) = 6.5 h.

B Quantification of terminal phenotypes of cells in (A).

C HeLa cells transfected with the indicated siRNAs were treated with taxol (200 nM) or nocodazole (5 μM) and monitored by time lapse microscopy. The cumulative percentage of cells in mitosis is plotted against the time in mitosis, with t_{50}(siLuc+NOC) = 6.1 h,

t_{50}(siP31-1+NOC) = 3.6 h, t_{50}(siLuc+TAX) = 6.2 h, t_{50}(siP31-1+TAX) = 3.1 h. Δt_{50}(siLuc–siP31-1/TAX) = –2.6 ± 0.4 h, n=6.

D Parental HeLa cells or HeLa cells stably transfected with siRNA resistant $p31^{comet}$ (eGFP-p31-siR) transfected with the indicated siRNAs were treated with taxol and monitored by time lapse microscopy. The cumulative percentage of cells in mitosis is plotted against the time in mitosis. Only cells with terminal apoptosis phenotype are included. A representative example of two experiments.

E Lysates of cells in (D) were blotted with anti-$p31^{comet}$ or anti-tubulin antibodies.

Figure S3 Noxa depletion delays apoptosis and increases adaptation during taxol-induced mitotic arrest.

A HeLa cells transfected with siControl or siNoxa-1 were synchronized by double thymidine block and released into medium with taxol. Samples were taken at the indicated timepoints after thymidine release and blotted with the indicated antibodies.

B DIC images of representative siLuc and siNoxa-1 cells undergoing mitotic arrest in taxol. The time in each frame indicated the time after nuclear envelope breakdown (NEBD).

C HeLa cells transfected with the indicated siRNAs, incubated with taxol and monitored by live cell imaging. Cumulative percentages of cells in mitosis plotted against their mitotic duration. Δt_{50}(siNoxa-1–siLuc) = 11.1 ± 4.9 h, n=4; Δt_{50}(siNoxa-2–siLuc) = 5.4 ± 0.3 h, n=2.

D Quantification of terminal phenotypes of cells in (D).

Figure S4 Mcl1 inhibits mitotic apoptosis.

A Lysates of parental HeLa cells and HeLa cells stably expressing Myc-Mcl1 with a doxycycline-inducible promoter were blotted with anti-Mcl1 and anti-Apc2 (as a loading control). The positions of Myc-Mcl1 and the endogenous Mcl1 are indicated.

B HeLa cells expressing inducible Myc-Mcl1 were cultured without or with 2 µg/ml doxycycline (Dox), synchronized by double thymidine arrest, and released into medium containing taxol. Samples were taken at the indicated timepoints after release from thymidine arrest and processed for FACS. The percentage of mitotic cells (MPM2-positive cells) is plotted against time from release.

C The percentage of apoptotic cells (cells with less than 2C DNA content) in (B) is plotted against time from release.

D Parental HeLa cells or HeLa cells expressing Myc-Mcl1 treated with Dox were synchronized by thymidine block, released into medium with 500 nM nocodazole, and monitored by time lapse microscopy. The cumulative percentage of cells in mitosis is plotted against the time in mitosis. t_{50}(Parental) = 8.8 h, t_{50}(Myc-Mcl1) = 14.2 h.

E Quantification of terminal phenotypes of cells in (D).

F HeLa cells transfected with siControl or siMcl1 were treated with taxol and monitored by time lapse microscopy. The cumulative percentage of cells in mitosis is plotted against the time in mitosis. Δt_{50}(siMcl1–siControl) = –4.7 ± 0.7, n=3.

G Quantification of terminal phenotypes of cells in (F).

H U2OS cells were transfected with the indicated plasmids, treated with taxol and monitored by time lapse microscopy. The cumulative percentage of cells in mitosis is plotted against the time in mitosis.

Figure S5 p31^{comet} antagonizes Noxa.

A Cumulative percentage of mitotic U2OS cells transfected with the indicated siRNAs and treated with taxol is plotted against their mitotic durations. A representative example of two experiments.

B Quantification of the terminal phenotypes of the mitotic cells in (A).

C Cumulative percentage of mitotic HeLa cells transfected with the indicated siRNAs and treated with taxol is plotted against their mitotic durations.

D Quantification of the terminal phenotypes of cells in (C).

Figure S6 Drp1 inhibition delays mitotic adaptation and prevents aberrant cytokinesis.

A U2OS cells synchronized by single thymidine block were collected by shake-off 15 h after release (3 h after taxol+/- Mdivi-1 addition), and further cultured in the taxol medium for the indicated times in the absence (left) or presence of 50 μM cycloheximide (CHX) (middle) or 5mM sodium azide and 2mM 2-deoxyglucose (2-DG) (right), stained with MPM2 antibodies and propidium iodide (PI), and subjected to FACS analysis.

B Selected DIC images from the time lapse microscopy of U2OS cells arrested in taxol and treated with cycloheximide with or without Mdivi-1. The daughter cells after undergoing adaptation are outlined in the bottom micrographs.

C Quantification of the aberrant cytokinesis phenotypes observed during mitotic adaptation of cells in (B).

D U2OS cells transfected with the indicated siRNAs for 48h and incubated with taxol for 15 h were collected by shake-off and further cultured in the taxol medium with or without 50

μM cycloheximide for 5 h, stained with MPM2 antibodies and PI, and subjected to FACS analysis. Representative FACS profiles shown.

Supplementary Tables

Table S1 Hits that increase cell viability in taxol by 2 standard deviations above the mean.

Table S2 Hits that decrease cell viability in taxol by 1.5 standard deviations below the mean.

Table S3 The top 38 hits in the secondary screen with the QIAGEN siRNAs.