SUPPLEMENTAL FIGURES

Figure S1. IR dose-dependent G_2/M arrest in HeLa cells.

HeLa cells were treated with different doses of IR and incubated for 28 h. The cells were then fixed, stained with propidium iodide, and subjected to flow cytometry analysis. The positions of 2N and 4N DNA contents are indicated.

Figure S2. Inhibition of cytokinesis does not affect mitotic catastrophe.

(A) Chromosomal segregation defects during UCN-01-mediated checkpoint abrogation. HeLa cells were treated with IR, incubated for 16 h, before UCN-01 was added. After 5 h, the cells were fixed and stained for DNA (Hoechst 33258), CREST (Alexa Fluor-488), and α-tubulin (Alexa Fluor-594). Representative images of cells at various stages of anaphase, telophase, and cytokinesis are shown. Merged images are shown on the right; the arrows indicate lagging chromosomes and chromosomal fragments; scale bars=10 μm.

(B) Aurora B inhibitor does not enhance mitotic catastrophe. HeLa cells expressing histone H2B-GFP and mRFP1-APC/C biosensor were irradiated, grown for 16 h, before treated with UCN-01. The cells were treated with control buffer or Aurora B inhibitor (ZM-447439), and subjected to time-lapse microscopy for 12 h. Key: grey=interphase; orange=mitosis (defined as from DNA condensation to decondensation); truncated bars=cell death; and blue=mitosis of cells that underwent mitotic catastrophe.

(C) DCB does not enhance mitotic catastrophe. The experiment was performed as in panel B except that the cells were treated with either control buffer or DCB before imaging. Key: red=mitosis (defined as from DNA condensation to anaphase onset).

(D) Inhibition of Aurora B does not enhance mitotic catastrophe. Cells were irradiated, treated with Aurora B inhibitor (AURKBi) or DCB, before incubated with UCN-01 as in panels B and C. The fates of the cells were analyzed by time-lapse microscopy (n=50). Cells that were able to complete mitosis (mitosis), die during mitosis (mitotic catastrophe), or did not enter mitosis throughout the imaging period (interphase) were quantified (mean±SD of three independent experiments).

Figure S3. p31^{comet} can bind to MAD2 and disrupt the spindle-assembly checkpoint.

(A) Interaction between p31^{comet} and MAD2. FLAG-p31^{comet} was expressed in a stable cell line by growing cells in medium without doxycycline for 48 h. Cell-free extracts were prepared and subjected to immunoprecipitation using normal rabbit serum (NRS), antiserum against FLAG, or the M2 anti-FLAG monoclonal antibody. The immunoprecipitates were immunoblotted with antibodies against FLAG or MAD2.

(B) Expression of p31^{comet} uncouples the spindle poison-induced checkpoint. The p31^{comet} stable cell line was treated with nocodazole in the presence (-p31^{comet}) or absence (+p31^{comet}) of doxycycline for 48 h. The cells were then harvested and analyzed with flow cytometry.

Figure S4. Functional rescue of mitotic catastrophe by MAD2.

(A) Mitotic catastrophe can be restored with MAD2. HeLa cells were transfected with control vector (n=32), plasmids expressing MAD2 shRNA (n=36), or MAD2 shRNA and MAD2 together (called pKAR-MAD2) (n=31). A YFP-expressing plasmid was co-transfected as a marker. The cells were irradiated, grown for 16 h, followed by UCN-01

treatment. Time-lapse microscopy was used to track individual cells for 12 h. The percentage of transfected cells that underwent mitotic catastrophe was quantified (n=42). **(B)** Mitotic length can be restored with MAD2. The experiment was performed as in panel A. The duration of mitosis was analyzed (mean±90% confidence interval). **(C)** Downregulation of MAD2 or overexpression of $p31^{comet}$ significantly reduces the duration of mitosis. HeLa cells expressing histone H2B-GFP were transfected with control vector, plasmids expressing FLAG-$p31^{comet}$ or MAD2 shRNA together with a mRFP1 marker. The cells were either untreated or irradiated, grown for 16 h, followed by UCN-01 treatment. Time-lapse microscopy was used to track individual cells for 12 h. The duration of mitosis (from DNA condensation to anaphase) of transfected cells was quantified. The mean and 90% confidence interval of the duration of mitosis in control cells, MAD2 shRNA-, or $p31^{comet}$-expressing cells in the absence (n=30, 60, and 36, respectively) or presence of IR and UCN-01 (n=35, 49, and 35, respectively) are shown.

Figure S5. Expression of $p31^{comet}$ attenuates mitotic catastrophe in cells treated with 40 Gy of IR and UCN-01.

HeLa cells expressing histone H2B-GFP were transfected with plasmids expressing FLAG-$p31^{comet}$ together with a YFP marker. The cells were irradiated with 40 Gy of IR, grown for 16 h, before treated with UCN-01. Time-lapse microscopy was used to track individual cells for 12 h (n=50). Key: grey=interphase; red=mitosis; blue=mitotic catastrophe; truncated bars=cell death.

Figure S6. Depletion of CDC20 potentiates mitotic catastrophe.

(A) Depletion of CDC20 or p31^{comet} lengthens mitosis. HeLa cells expressing histone H2B-GFP and mRFP1-APC/C biosensor were transfected with control siRNA (n=58), siRNA against CDC20 siRNA (n=30), or p31^{comet} siRNA (n=39). The length of mitosis of individual cells was analyzed by time-lapse microscopy (mean±90% confidence interval).

(B) Depletion of CDC20 potentiates mitotic catastrophe. HeLa cells were transfected with control siRNA or CDC20 siRNA. After 6 h, the cells were irradiated, grown for 16 h, and treated with UCN-01 as indicated. After 6 h, viability was determined with trypan blue exclusion assay (mean±SD of three independent experiments).

Figure S7. Different cell lines display different susceptibility to mitotic catastrophe.

Different cell lines (H1299, HCT116, U2OS, and NIH3T3) expressing histone H2B-GFP were irradiated with either 15 Gy or 40 Gy of IR. The cells were then grown for 16 h or 24 h (for U2OS and NIH3T3) before either mock-treated or treated with UCN-01. Individual cells were tracked with time-lapse microscopy for 12 h (n=50). Key: grey=interphase; red=mitosis; blue=mitotic catastrophe; truncated bars=cell death.

Figure S8. Mitotic catastrophe can be promoted in U2OS cells by CDC20 siRNA.

U2OS cells expressing histone H2B-GFP were transfected with either control or CDC20 siRNA. The cells were irradiated with either 15 Gy or 40 Gy of IR, grown for 24 h, before treated with UCN-01. Individual cells were tracked with time-lapse microscopy for 24 h (n=50). Key: grey=interphase; red=mitosis; truncated bars=cell death.

SUPPLEMENTAL VEDIOS

Video 1. HeLa cells treated with IR.

HeLa cells expressing histone H2B-GFP and mRFP1-APC/C biosensor were irradiated and grown for 16 h. The cells were subjected to time-lapse microscopy. Channels for bright field (left), histone H2B-GFP (middle), and mRFP1-APC/C biosensor (right) of representative cells are shown.

Video 2. Cells treated with IR and UCN-01.

HeLa cells expressing histone H2B-GFP and mRFP1-APC/C biosensor were irradiated and grown for 16 h. The cells were then treated with UCN-01 and subjected to time-lapse microscopy. Channels for bright field (left), histone H2B-GFP (middle), and mRFP1-APC/C biosensor (right) of representative cells are shown.

Video 3. IR and UCN-01-induced mitotic catastrophe.

HeLa cells expressing histone H2B-GFP and mRFP1-APC/C biosensor were irradiated and grown for 16 h. The cells were then treated with UCN-01 and subjected to time-lapse microscopy. Channels for bright field (left) and histone H2B-GFP (right) of representative cells are shown.

Video 4. Cells undergoing abortive cytokinesis.

HeLa cells expressing histone H2B-GFP and mRFP1-APC/C biosensor were irradiated and grown for 16 h. The cells were then treated with UCN-01 and subjected to time-

lapse microscopy. Channels for bright field (left), histone H2B-GFP (middle), and mRFP1-APC/C biosensor (right) of representative cells are shown.

Video 5. Cells treated with the Aurora B inhibitor ZM-447439.

HeLa cells expressing histone H2B-GFP and mRFP1-APC/C biosensor were irradiated and grown for 16 h. The cells were then treated with UCN-01, ZM-447439, and subjected to time-lapse microscopy. Channels for bright field (left), histone H2B-GFP (middle), and mRFP1-APC/C biosensor (right) of representative cells are shown.

Video 6. Cells treated with DCB.

HeLa cells expressing histone H2B-GFP and mRFP1-APC/C biosensor were irradiated and grown for 16 h. The cells were then treated with UCN-01, DCB, and subjected to time-lapse microscopy. Channels for bright field (left), histone H2B-GFP (middle), and mRFP1-APC/C biosensor (right) of representative cells are shown.

Video 7. IR and UCN-01-treated p31^{comet}-expressing cells.

HeLa cells expressing histone H2B-GFP were transfected with plasmids expressing FLAG-p31^{comet} together with a mRFP1 marker. The cells were irradiated with IR and grown for 16 h. The cells were then treated with UCN-01 and subjected to time-lapse microscopy. Channels for bright field (left) and histone H2B-GFP (right) of representative cells are shown.

Video 8. IR and UCN-01-treated MAD2 shRNA-expressing cells.

HeLa cells expressing histone H2B-GFP were transfected with plasmids expressing MAD2 shRNA together with a mRFP1 marker. The cells were irradiated with IR and grown for 16 h. The cells were then treated with UCN-01 and subjected to time-lapse microscopy. Channels for bright field (left) and histone H2B-GFP (right) of representative cells are shown.

Video 9. IR and UCN-01-treated CDC20-depleted cells.

HeLa cells expressing histone H2B-GFP and mRFP1-APC/C biosensor were transfected with CDC20 siRNA. The cells were irradiated with IR, grown for 16 h, treated with UCN-01 and subjected to time-lapse microscopy. Channels for bright field (left) and histone H2B-GFP (right) of representative cells are shown.

Video 10. IR and UCN-01-treated p31^{comet}-depleted cells.

HeLa cells expressing histone H2B-GFP and mRFP1-APC/C biosensor were transfected with p31^{comet} siRNA. The cells were irradiated with IR, grown for 16 h, treated with UCN-01 and subjected to time-lapse microscopy. Channels for bright field (left) and histone H2B-GFP (right) of representative cells are shown.