Legends to Supplementary Figures:

**Supplementary Figure S1:**
MCF-7 cells were infected with pLL-GFP or pLL-Cdh1 and selected with puromycin. Cells were grown on glass coverslips, fixed with 3.7% formaldehyde in PBS and stained with anti-p53 and DAPI. Ten microscopic fields were counted for p53 positivity. Averages and standard deviations of 10 fields of cells are indicated.

**Supplementary Figure S2:**
A. Schematic representation of the FUCCI system, which was used in the experiments depicted in Figures 2C and 2D. In these cells, fragments of geminin and Cdt1 are tagged with fluorescent reporters, which allow the discrimination of G₁ and S/G₂ cells. B/C. U2OS-FUCCI cells treated as described in Figure 2C were prepared for Rad51 (panel B) and 53BP1 (panel C) immunofluorescence. Shown are representative images of the different time-points. Scale bars correspond to 20 µm.

**Supplementary Figure S3:**
A. Indicated are established substrates of the APC/C<sup>Cdh1</sup> that were identified in our mass spectrometry analysis. Log2-transformed ratios between t=2.5 and t=0 hours after release from nocodazole-mediated mitotic arrest are indicated. Literature references to these APC/C<sup>Cdh1</sup> are provided in Table S3.
B. Proteins that were downregulated at least 1.0 (log2-transformed SILAC ratios) and were part of the ‘DNA Damage response’ (see Supplementary Table S2), were queried for the presence of KEN or D-box destruction motifs. To this end, GPS-ARM software (Liu et al, 2012) was used to identify D-boxes and KEN boxes. Open orange squares represent any D-box, whereas filled orange squares represent an optimal D-box. Likewise, open green squares represent any KEN box, whereas filled green squares represent optimal KEN box motifs. Subsequently, protein sequences from orthologs from indicated species were aligned using Clustal Omega (www.ebi.ac.uk/Tools/msa/clustalo/) using default settings. Boxes indicate evolutionary conserved destruction motifs. Grey filled boxes indicated evolutionary conservation of an optimal destruction motif. Numbers above destruction motifs indicate the amino acid location of the start position of the destruction motif in the human sequence.

Supplementary Figure S4:
A. RPE-1 cells were treated for 18 hours with S-Trityl-L-Cysteine (STLC, 5 µM) and mitotic cells were collected by shake-off. Mitotic cells were subsequently washed with warm culture medium and subsequently replated. Total cell lysates were immunoblotted with anti-CtIP, anti-Plk1 and anti-Actin. In parallel to obtaining total cell lysates, cells were fixed in ethanol and stained for the mitotic marker MPM-2. Representative plots are shown. B. HeLa cells were left untreated (asynchronous, “AS”) or were treated with nocodazole (75 ng/ml) for 14 hours. Subsequently, cells were lysed in RIPA buffer, containing 0.1% SDS; PMSF and benzamidine. Next, the samples of asynchronous or nocodazole-treated cells were incubated for 30 minutes in the absence or presence of λ-phosphatase (“λ-PPase”). Control samples treated with a combination of λ-PPase and inhibitors (50 mM EDTA and 10 mM sodium orthovanadate) were included. C. U2OS cells were transfected with indicated siRNAs and total cell lysates were made at 48 hours after transfection. Western blotting was performed with indicated antibodies. In parallel, cells were fixed in ethanol, stained with PI/RNAse and subsequently analyzed by flow cytometry. D. RPE-1 cells were treated as described for Figure 4D. In parallel to making total cell lysates for Western blotting, cells were fixed in ethanol and stained for anti-phospho-HistoneH3/Alexa-488 in combination with PI/RNAse. Representative DNA profiles and phospho-
Histone-H3 plots are shown. Numbers indicate averages and standard deviations of phospho-Histone-H3 content from three independent experiments. E. RPE-1 cells were treated as for Figure 4F. In parallel to obtaining total cell lysates, cells were fixed in ethanol and stained for phospho-Histone-H3. Representative plots are shown.

**Supplementary Figure S5:**

**A.** HEK293 Flp-In T-REx cells were induced to express GFP-CtIP-wt or GFP-CtIP-K467A using doxycycline. Cell lysates were used for Cdh1 immunoprecipitations. Total cell lysates (‘input’) and Cdh1 immunoprecipitations (‘anti-Cdh1 IP’) were immunoblotted for indicted proteins. **B.** Polyclonal U2OS Flp-In T-REx cells were induced to express GFP-CtIP-wt (upper panels) or GFP-CtIP-KA (middle panels) using doxycycline for 24 hours. Subsequently, cells were synchronized using a single thymidine block. After release from the thymidine treatment, cells were treated with nocodazole (250 ng/ml) and at indicated time points, cells were harvested, fixed in ethanol and stained for the mitotic marker MPM-2. Lower panels: Cells were treated as for upper and middle panels. At the moment of thymidine wash-out, cells were fixed in ethanol and levels of GFP-CtIP-wt or GFP-CtIP-KA were assessed using flow cytometry. Black lines indicate untreated cells, green lines indicate doxycycline-treated cells.

**Supplementary Figure S6:**

**A.** U2OS cells were synchronized for 18 hours using thymidine (2 mM). Cells were then released from the S-phase block to allow progression towards G2. At 7 hours post-release, cells were treated with doxorubicin (‘dox’, 0.5 μM) for 1 hour. Subsequently, fresh (dox-free) medium was added, and cells were left untreated or were treated with proTAME (‘PT’, 20 μM) for 5 hours. At indicated time points, cells were fixed and analyzed for cell cycle distribution. **B.** Same cells as in panel A were lysed in RIPA buffer at indicated time points. Whole cell extracts were analyzed by immunoblotting with indicated antibodies. (‘short’) and (‘long’) depict short and long exposures of the same membrane, respectively. ‘*’ indicate phosphorylated species of proteins. **C.** U2OS Flp-In T-REx cells were induced to express GFP-CtIP-wt using doxycycline (1μg/ml) for 24 hours. Subsequently, cells were synchronized using a single thymidine block. At 8 hours after release, cells were treated with DMSO or
proTAME (12 μM), subsequently irradiated with 10 Gy, and imaged using fluorescence time-lapse microscopy. Representative stills of GFP and DIC movies are indicated.

**Supplementary Figure S7:**

**A.** Polyclonal U2OS Flp-In T-REx cells were induced to express GFP-CtIP-wt or GFP-CtIP-K467A using doxycycline. Lysates were immunoblotted using anti-CtIP and anti-TFIH. In parallel, U2OS Flp-In T-REx cells were induced to express GFP-CtIP-wt or GFP-CtIP-K467A and subsequently micro-irradiated. Cells were then fixed and stained for γ-H2AX to visualize laser-induced DNA damage. **B.** Monoclonal U2OS-GFP-CtIP-wt were transfected with CtIP siRNA, and if indicated, cells were induced to express GFP-CtIP-wt using doxycycline (0.5 μg/ml). Cells were irradiated with 10 Gy and harvested after 1 or 4 hours. Total cell lysates were immunoblotted with indicated antibodies. ‘*’ indicates phosphorylated species of proteins. **C.** Monoclonal U2OS-GFP-CtIP-wt or U2OS-GFP-CtIP-K467A cells were transfected with indicated siRNA. If indicated, cells were induced to express GFP-CtIP-wt or GFP-CtIP-K467A using doxycycline (0.5 μg/ml and 1 μg/ml respectively). 24 hours after transfection. At 48 hours after transfection, cells were harvested, and total cell lysates were immunoblotted for CtIP and Actin. ‘Short ex’ indicates short exposure and ‘long ex’ indicates long exposure. In parallel, cells were irradiated (2 Gy) at 48 hours after transfection, and nocodazole was added 1.5 hours after irradiation to trap cells in mitosis. Samples were fixed in ethanol at 4 hours and 8 hours after irradiation, stained with the mitotic marker MPM-2 in combination with propidium iodide/RNAse treatment and subsequently analyzed by flow cytometry. Percentages of mitotic cells are indicated. Averages and standard deviations of three experiments are indicated. **D.** U2OS cells were transfected with siCTRL or siCdh1 and treated as for panel C. Lysates were blotted using anti-Cdh1 and anti-Actin.

**Supplementary Figure S8:**

**A-C.** Monoclonal U2OS-GFP-CtIP-wt, U2OS-GFP-CtIP-K467A and U2OS-GFP-CtIP-T847A cells were transfected with siCtIP and were induced to express GFP-CtIP-wt (0.5 μg/ml doxycycline), GFP-CtIP-K467 or GFP-CtIP-T847A (1 μg/ml
doxycycline) at 24 hours after transfection, At 48 hours after transfection, cells were trypinized and replated for clonogenic survival assays. For panel A, cells were irradiated as in Figure 2A. For panels B and C, cells were treated with indicated doses of doxorubicin. D. FACS profiles referring to the experiment shown in Figure 8F. In parallel to making total cell lysates for Western blotting, cells were fixed in ethanol, stained with DAPI and subjected to flow cytometry.

**Supplementary Data S1:**
Proteins identified using SILAC mass spectrometry, as depicted in Figure 3D.

**Supplementary Table S1:**
List of Gene Ontology (GO) classifiers that were used to select DNA Damage Response components from the SILAC mass spectrometry data, as depicted in Figure 3F.

**Supplementary Table S2:**
List of primary antibodies used in this study.

**Supplementary Table S3:**
Literature references to APC/C^Cdhh1 targets as listed in Figure S3A.