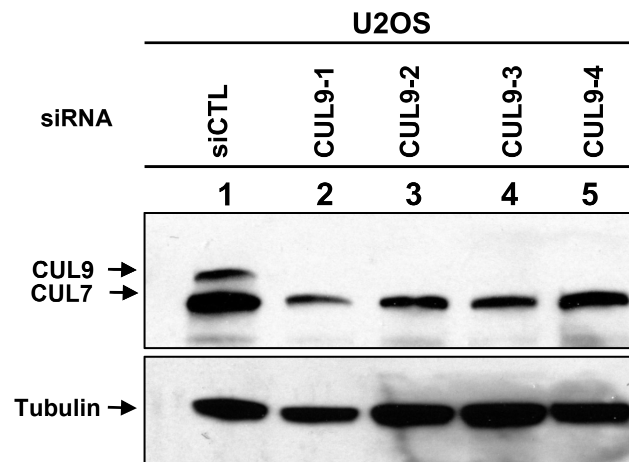


Figure S1, related to Figure 2. CUL9 functions to maintain genome integrity.

Four different siRNA oligonucleotides against CUL9 were synthesized and transfected to U2OS cells. The knocking down efficiency was determined by direct immunoblotting using an antibody that recognizes both CUL7 and CUL9 (Sigma, AB38).

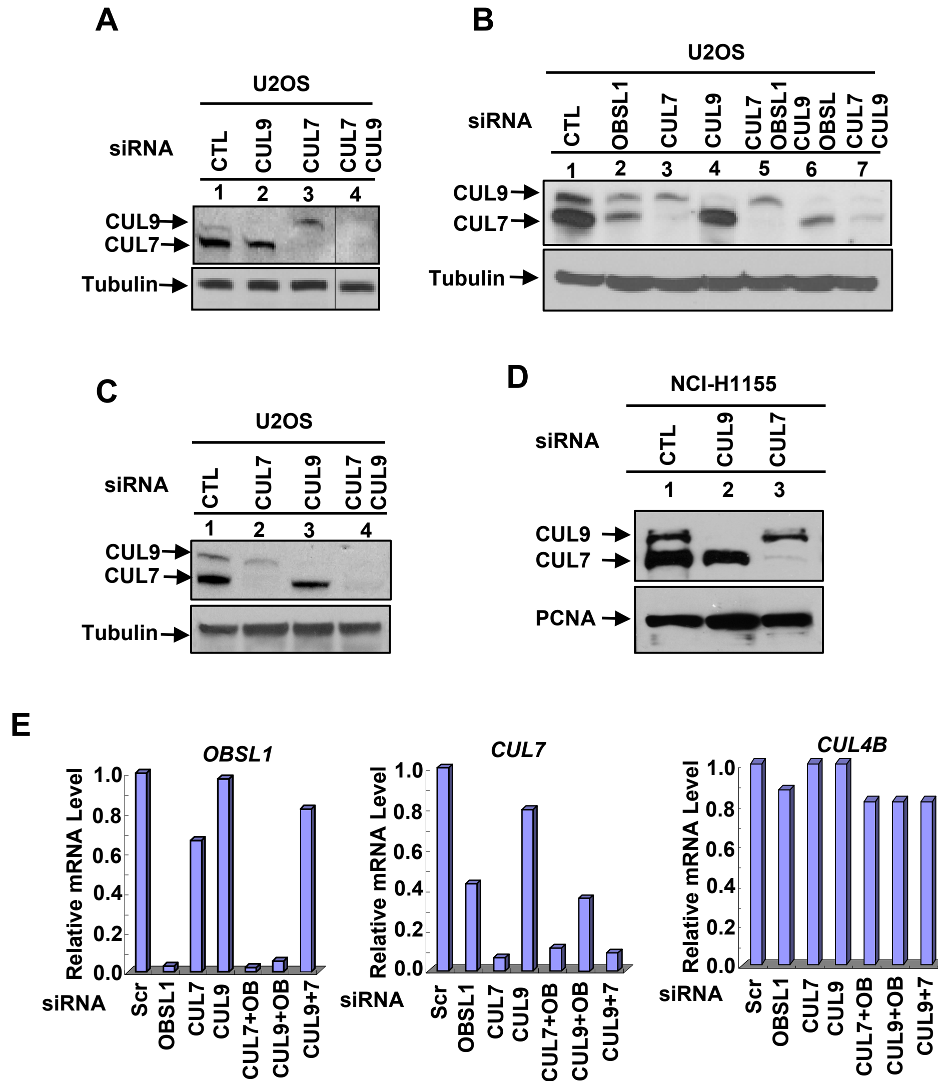


Figure S2, related to Figure 3. CUL9 is a downstream effector of CUL7 and OBSL1. (**A, B, C, D**) The knocking down efficiencies of different genes were verified by direct immunoblotting correspond to main Figures 3A, 3B, 3C and 3D, respectively.
(**E**) Knocking down efficiency in main Figure 3B was determined by Q-PCR.

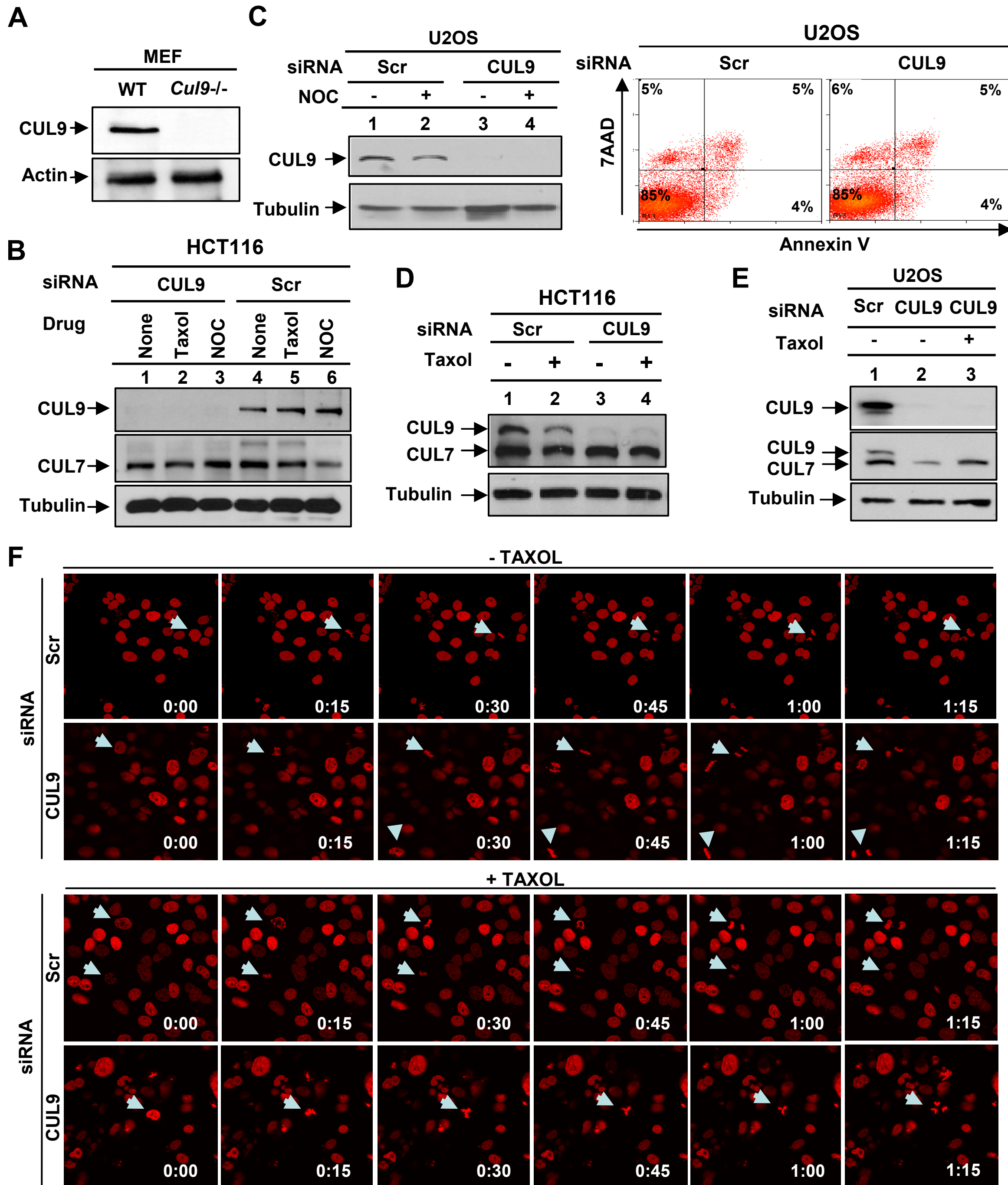


Figure S3, related to Figure 4. Loss of CUL9 function causes non-apoptotic cell death after microtubule damage, (A, B). Knockout and knockdown of CUL9 in main figure 4A and 4B were confirmed by direct immunoblotting. **(C)** U2OS cells were transfected with indicated siRNA oligos for 48 hours. The knockdown efficiency was determined by direct immunoblotting. Cells were then collected separately, stained with Annexin V and 7AAD and analyzed by FACS. (**D and E**) The knocking down efficiencies in main figure 4E and 4F were verified by direct immunoblotting. **(F)** A culture of HCT116 cells expressing mCherry fluorescent proteins fused to human histone H2B. The video was captured and imaged by confocal laser scanning microscopy. Chromosomes are visualized by H2B-mCherry (red). Time is in hour:min.

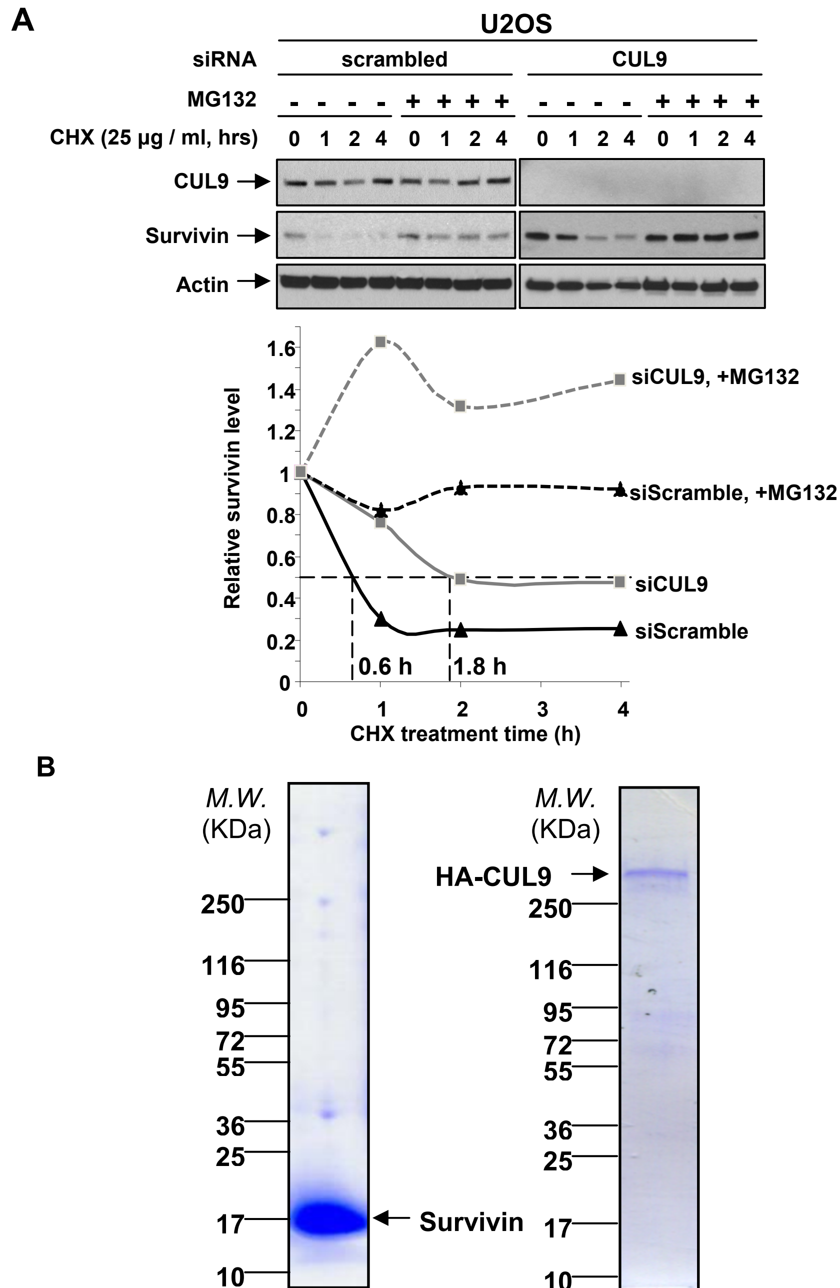
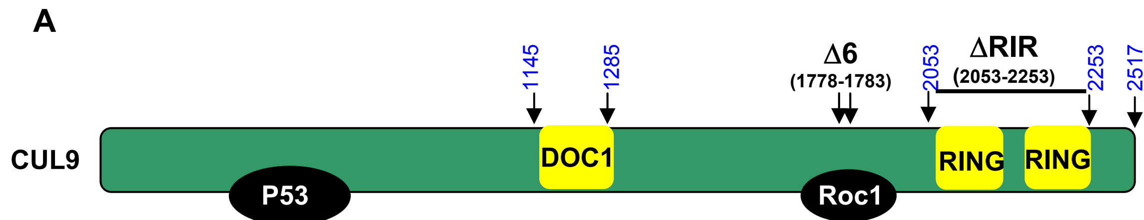


Figure S4, related to Figure 5. Survivin is a substrate of CUL9 E3 ligase

(A) The half-life of Survivin protein was increased in U2OS cells depleted for CUL9. (B) HA-CUL9 E3 ligase complex was purified from transfected 293T cells and eluted with HA antigen peptide, and recombinant survivin was purified from bacteria. Purified E3 complex and substrate were used for in vitro ubiquitylation assay in Figure 6G and 6H.

A



B

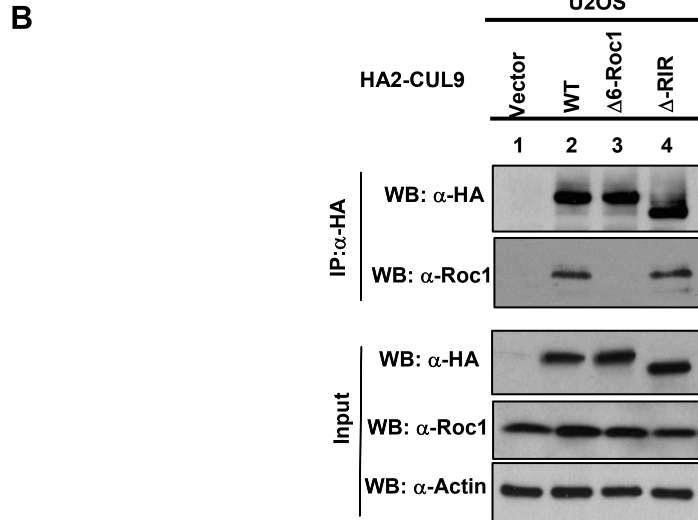


Figure S5, related to Figure 6. CUL7 forms heterodimer with CUL9 to inhibit CUL9

(**A**) Schematic representation of human CUL9 protein. The mutations made for functional analysis in main figure 6 are indicated.

(**B**) U2OS cells were transfected with HA tagged CUL9 or CUL9 mutants. Two days after transfection, expression and association between CUL9 and ROC1 were determined by western or IP-western analyses.

Supplemental Experimental Procedures

Plasmids

Most *CUL9* plasmids were described in (Pei et al., 2011). Mutations were introduced by site-directed mutagenesis (Stratagene) and verified by DNA sequencing. pEGFP-Ubiquitin and pEGFP-EB1 were purchased from Addgene. pMSCV-α-tubulin was a gift from Dr. James Bear. pcDNA-Flagx3-Survivin and pET-Survivin were constructed by cloning the full length human survivin cDNA into pcDNA3-Flag3 and pET31 vector.

siRNA and primer sequences

All siRNA oligos were purchased commercially from Invitrogen. siRNA, Stealth RNAi™ siRNA Negative Controls LO GC (12935-200) was used as a negative control. The sequences of siRNA oligo and primers used for RT-qPCR for CUL7, CCDC8 and OBSL1 were described in the accompanying paper. The siRNA oligo sequences for CUL9 are: siCUL9-1 GCUGAGAGACACGUUGUUUAG; siCUL9-2 GCUGAAUAAAGGUCUCUUUCU; siCUL9-3 UACUGAGGGUGCUCUUCUG.

For RT-qPCR analysis, total RNA was extracted from cells with RNeasy kit (Qiagen), and 1 μg of RNA was primed with Random Hexamer primers (Invitrogen) for cDNA synthesis. The cDNA was added to a real time qPCR mixture that contained 1× SYBR Green PCR master mix (Applied Biosystems) and 150 nM gene-specific primers. Assays were performed in triplicate on a 7900 HT sequence detection system (Applied Biosystems). The expression level of each gene was normalized by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels.

Specific qPCR primers are: CUL9 F-AACCCTGGAACAGAAGAG and CUL9 R-GAGAGGACATCTGTACTGG; Survivin F-TGCTTCAAGGAGCTGGAAGG and Survivin R: AAGTGGTGCAGCCACTCTGG.

Antibodies and immunological procedures

Antibodies used in this study are rabbit polyclonal anti-CUL9 (H7-AP1 A300-098A), anti-CUL7 (A300-223A) and anti-CCDC8 (A302-990A) from Bethyl; mouse monoclonal anti-HEC1 (clone 9G3), anti-Aurora B (clone 3609), anti-γ-tubulin (clone TU-30), and rabbit polyclonal anti-α-tubulin (ab15246), anti-pericentrin (ab4448) from Abcam; monoclonal anti-p53 (clone DO-1) from Santa Cruz; rabbit polyclonal anti-Aurora B (phosphor T232) from Rockland; mouse monoclonal anti-FLAG M2 from Sigma.

Cell culture, transfections and flow cytometry

U2OS cells were maintained in McCoy's 5A with 10% FCS; HEK293T and MEFs were maintained in DMEM with 10% FCS; Fibroblasts from 3M patients were maintained in DMEM with GlutaMax and 10% FCS; all cells were incubated at 37 °C, 5% CO_2. Plasmid transfections were performed using FuGene 6 (Roche), and siRNA oligos were transfected with Lipofectamine 2000 (Invitrogen), following the manufacturers' instructions.

For cell cycle analysis, after trypsinization and PBS wash, cells were fixed by cold ethanol for at least 3 hours. Total DNA was stained by Propidium Iodide (PI). For apoptosis or cell death analysis, cells were harvested by trypsinization, stained with

Annexin V and 7AAD, and then analyzed by FACS. FACS analysis was performed using the CYAN ADP Analyzer (Dako, Fort Collins, Colorado). Data analysis was performed using Summit v4.3 software (Dako, Fort Collins, Colorado).

Immunofluorescence

U2OS cells were fixed for 10 min in 4% paraformaldehyde and subsequently permeabilized for 5 to 10 min with 0.2% Triton X-100. Cells were washed in PBS and then incubated for 30 min in PBS with 1% BSA. Cells were subsequently incubated for 1.5 h with indicated primary antibodies, followed by secondary antibody incubation for 40 min (Jackson Lab). Nuclei were stained with 4',6-diamidino-2-phenyindole (DAPI). Cells were then covered with 80% glycerol and fluorescence was observed and pictured by Olympus inverted microscope with fluorescence illumination source or FV1000 confocal system. The photographs shown are representative of each experiment.

In vitro and *in vivo* ubiquitylation assays

For *in vitro* substrate independent ubiquitination assays, transfected HA-CUL9 immunocomplex was immunoprecipitated with anti-HA antibody from 293T cells, immobilized on protein A-agarose beads, and washed three times with an NP-40 lysis buffer and twice with a ligase assay buffer (25 mM Tris-HCl (pH 7.5), 50 mM NaCl, 1 mM EDTA, 0.01% NP-40, 10% glycerol). Immobilized immunocomplex was eluted by incubating with a molar excess of HA peptide. His tagged survivin protein was produced by bacteria. Different combinations of E1 (100 ng, Boston Biochem), E2 (100 ng, Boston

Biochem) and E3 (CUL9) were mixed with 100 ng of recombinant survivin substrate in an ubiquitin ligase reaction buffer [10 µg of bovine ubiquitin (Sigma), 2 mM ATP, 1 mM MgCl2, 0.3 mM dithiothreitol, 25 mM Tris-HCl (pH 7.5), 120 mM NaCl, 0.5 U of creatine phosphokinase (Sigma), and 1 mM phosphocreatine (Sigma)]. Reactions were carried out for 120 min at 37 °C, terminated by boiling for 5 min in a SDS sample buffer containing 0.1 M dithiothreitol, reaction products were resolved by SDS-PAGE and analyzed by immunoblotting with anti-survivin antibody. For *in vivo* ubiquitination assay, U2OS cells were plated in 100-mm dishes were transfected with plasmid expressing HA-ubiquitin and siRNA oligos as indicated. Twenty hours after transfection, cells were treated with the proteasome inhibitor MG132 (25 µM) for 4 h. Cells were harvested and suspended in 100 µl of SDS-lysis buffer [50 mM Tris-HCl (pH 7.5), 0.5 mM EDTA, 1% SDS, 1 mM DTT]. Samples were boiled for 10 mins and subsequently spun at 14,000 rpm, for 10 min. The supernatant was diluted 1:10 with 0.5% NP-40 buffer and immunoprecipitated with anti-survivin (1.0 µg). Immunoprecipitates were immobilized on protein A agarose beads, washed three times with 0.5% NP-40 buffer and resolved by 4-15% gradient SDS-PAGE, and analyzed by immunoblotting with anti-HA for HA-ubiquitin detection and other antibodies indicated in the Figure.