Legends for the Supplementary Movies

Movie S1
Related to main Figure 1E, the movie shows cell division of U2OS cells 48 hours after treated with scrambled siRNA. Pictures were taken in 5-minute intervals.

Movie S2
Related to main Figure 1F, the movie shows cell division of U2OS cells 48 hours after treated with siCUL7. Pictures were taken in 5-minute intervals. Note the long bridge between the daughter cells and their later death.

Movie S3
Related to main Figure 1G, the movie shows cell division of U2OS cells 48 hours after treated with siCUL7. Pictures were taken in 5-minute intervals. Note that one daughter cell entered next cell division while still connected to the other daughter cell.

Movie S4
Related to the left panel of main Figure 2B, the movie shows control cells which were transiently expressed with EGFP-EB1 to visualize microtubule plus ends. The movie shows the EB1 movement, with cells were imaged every 5 seconds.

Movie S5
Related to the right panel of main Figure 2B, the movie shows CUL7 depleted cells which were transiently expressed with EGFP-EB1 to visualize microtubule plus ends. The movie shows the EB1 movement, with cells were imaged every 5 seconds. Note the abnormal movement of microtubule plus ends.

Supplemental Experimental Procedures

Plasmids

pcDNA3-3×FLAG-CUL7 was previously described (Andrews, 2006). pHIT-3×Myc-CUL7 was obtained by inserting full length CUL7 cDNA into pHIT-3×Myc vector. Mutations were introduced by site-directed mutagenesis kit (Stratagene). pEGFP-Ubiquitin and pEGFP-EB1 were purchased from Addgene. pMSCV-α-tubulin was a gift from Dr. James Bear. pKMyc-OBSL1B was kindly provided by Dr. Mark Russell.pBABE-FLAG-SBP-FBXW8 was cloned by inserting the full length human FBXW8 cDNA into pBABE-FLAG-SBP vector.

siRNA sequences

All siRNA oligos were purchased commercially from Invitrogen. siRNA, Stealth RNAi™ siRNA Negative Controls LO GC (12935-200) was used as negative control. The siRNA oligo sequences are:

siCUL7-A: GACAGAACUCUAUGCUGUGCCUUAU,

siCUL7-B: GCACAUGUUGAGUAGUCCUGAUUAU,

siOBSL1-1: GAGCACGGGAUUGCCGUGCUGGAAU,

siOBSL1-3: CGGGCUGGAAGUGGAGGCAUCAGAU,

siCCDC8-A: GGGUCAGAGGUUACAGAUAAUCAAA,

siCCDC8-D: CAGUGCCCAUGUUUGAGGACAACCU

siCUL3: GUCGUAGACAGAGGCGCAA

siMAD2: GCCACUGUUGGAAGUUUCUUGUUCA

The underlined bases in all pHIT-3×Myc-CUL7 constructs were changed into “G”, causing silence mutations, to make the constructs resistant to siCUL7-A.

Antibodies and immunological procedures

Antibodies used in this study are rabbit polyclonal 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-Histone H3 (phosphor S10) (ab47297), anti-pericentrin (ab4448) from Abcam; anti-LAP2 (clone 27) from BD Transduction Laboratories; Monoclonal anti-p53 (clone DO-1) from Santa Cruz; mouse monoclonal anti-FLAG M2 from Sigma; rabbit polyclonal anti-ROC1. Rabbit polyclonal anti-MAD2 from Bethyl Laboratories.

For protein expression and binding assays, 293T cells were transiently transfected interested plasmids by using Fugene 6 transfection reagent (Roche). Forty-eight hours after transfection, cells were harvested and lysed for 1 h in NP-40 lysis buffer (50 mM Tris-HCl (pH7.5), 150mM Nacl, 0.5% NP-40, 50 mM NaF) containing protease inhibitor cocktails (Sigma). The cell lysates were centrifuged at 14, 000 x *g* at 4°C for 10 min. The supernatants were then incubated with antibody and Protein A or G agarose beads (Invitrogen) overnight. The beads were then washed three times with lysis buffer. The bound protein complexes were then solubilized in sample buffer and analyzed by western blot analysis using the antibodies indicated.

Quantitative RT-PCR

Total RNA was extracted from the cells with RNeasy kit (Qiagen), and 1 µg total RNA was primed with Random Hexamer primers (Invitrogen) for cDNA synthesis. The cDNA was added to a Q-RT–PCR 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 with mRNA amount of glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Specific PCR primers are:

CUL7 F: TCCCTTGCTCTGTGAAGGAC

CUL7 R: CTGGTACTCAGGATGCCCA

OBSL1 F: GGCGGCTTCGTGCTCAAGGT

OBSL1 R: GAACCAGGCCGTGGGGATGC

GAPDH F: AGGTGAAGGTCGGAGTCAAC

GAPDH R: GACAAGCTTCCCGTTCTCAG

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. WI-38 and NHF-1 cells were maintained in MEM with 10%FCS. All cells were incubated at 37ºC, 5% CO_2. Plasmid transfections were performed using FuGene 6 from Roche, and siRNA oligos were transfected with

Lipofectamine 2000 from Invitrogen, following the manufacturers' instructions. 3M fibroblasts, WI-38, and NHF-1 cells were collected and stained with Hoechst 33342, followed by FACS analysis.

Immunofluorescence

U2OS cells were fixed for 20 min in 4% paraformaldehyde and subsequently treated for 7 minutes with 0.2% Triton X-100. Cells were washed in PBS and then incubated for 15 minutes in PBS with 1% BSA. Cells were subsequently incubated for 1.5 hours with the indicated primary antibodies, followed by secondary antibody incubation for 40 minutes (Jackson Lab). Nuclei were stained with 4',6-diamidino-2-phenyindole (DAPI). Cells were then covered with 80% glycerol and fluorescence was observed microscopically. The photographs shown are representative for each experiment.