Supplement material and methods

Immunoprecipitation

Myc-tagged cullin 3, GST-tagged full length Clld7, GST-tagged RCC1 domain (from amino acid 40 to 299), GST-tagged BTB domain of Clld7 (from amino acid 371 to 460) were co-transfected into HEK 293 cells as indicated in supplement figure 3. Two days after transfection, cell lysates were prepared in protein lysis buffer containing 0.5% Nonidet P-40 (NP40), 150 mM NaCl, 50 mM Tris-HCl (pH 7.5), and protease inhibitor cocktail (Roche). Samples were then centrifuged at 13,000 rpm for 5 minutes. Then the supernatant was mixed with 20 µl glutathione-Sepharose 4B slurry (GE Healthcare) (previously equilibrated with lysis buffer) and incubated for 1hr at 4°C with rotation. The Sepharose beads were then washed three times with protein lysis buffer. Bound proteins were eluted with 2× SDS-PAGE sample buffer and analyzed on a 4-12% bis-Tris gradient gel (Invitrogen), followed by Western blotting with the indicated antibodies. The myc antibody (9E10) was purchased from Millipore (05-419).

Co-localization of phospho-CHK2 and γ-H2AX

For immunofluorescence experiments, cells were plated, fixed, and stained as previously described in the material and methods part. Cells were incubated with γ-H2AX antibody (Millipore, #05-636) and Phospho T68-Chk2 antibody (Cell signaling, #2661) at 4°C overnight. Alexa Fluor 488(Invitrogen, #A11001) donkey anti-mouse and Alexa Fluor 568 donkey anti-rabbit (Invitrogen, #A10042) labeled secondary antibody were used for detection. Slides incubated with secondary antibody only are used as negative controls. Nuclei were counterstained with Hoechst 33258 and Topro3. Immunofluorescence images were taken with a laser scanning Zeiss Axioskop PCM2000 confocal fluorescence microscope. Images used for comparison between different treatments were acquired with the same instrument settings and exposure time and were processed equally.