Yan et al. Supplementary Figure S1

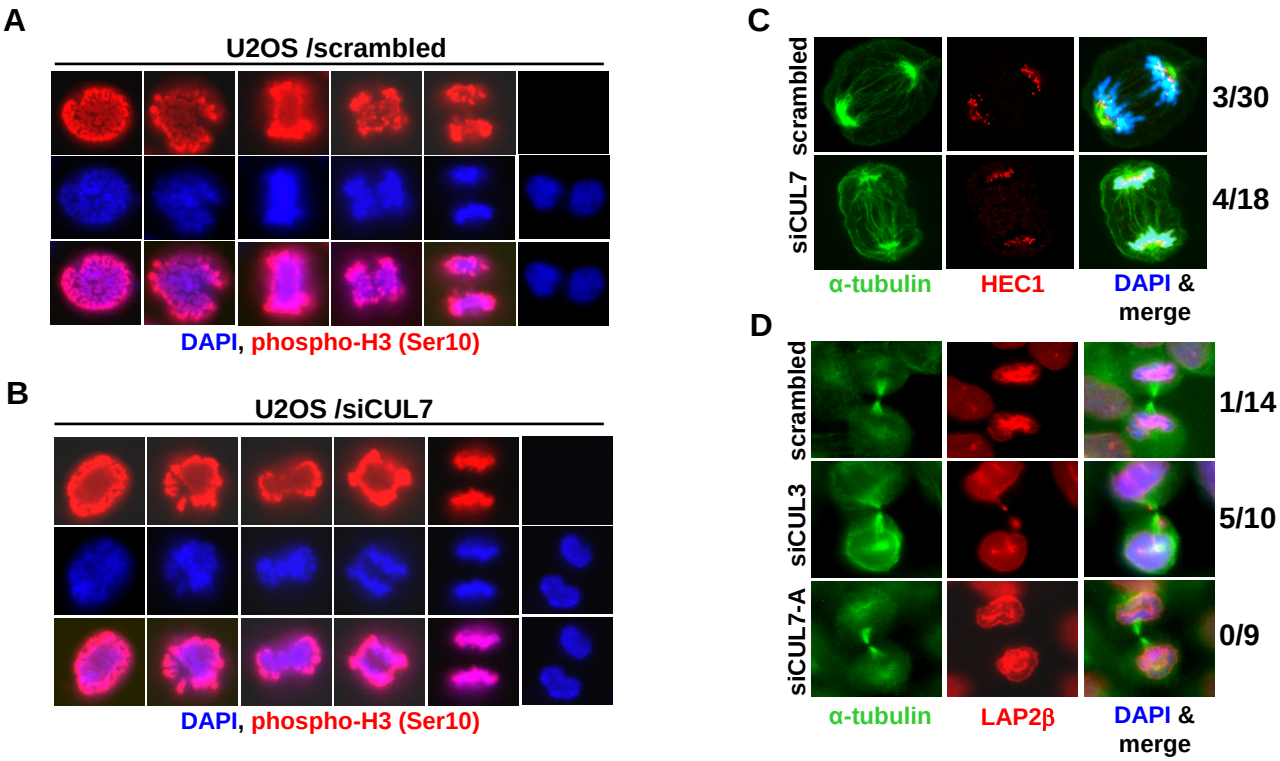

Figure S1. Knocking down Cul7 does not cause significant defect in chromosome condensation and decondensation, or significant lagging chromosomes, related to Figure 1.

(**A**, **B**) Control or CUL7 depleted cells were fixed and stained with anti-phospho H3 Ser10 and DAPI. Images show the cells at different phases of mitosis. (**C**) CUL7 depletion does not cause obvious lagging chromosomes. Control or CUL7 depleted cells were stained against α-tubulin and HEC1, and DAPI. Anaphase cells with lagging chromosomes were scored. (**D**) Cells were treated with scrambled, CUL3, or CUL7 siRNA, and then stained with antibodies against α-tubulin and LAP2β, and DAPI. LAP2β is an inner nuclear membrane protein, staining of which helps to identify lagging chromosomes. siCUL3, which depletes CUL3 causes significant accumulation of anaphase cells with lagging chromosome, was included as a positive control.

Yan et al. Supplementary Figure S2

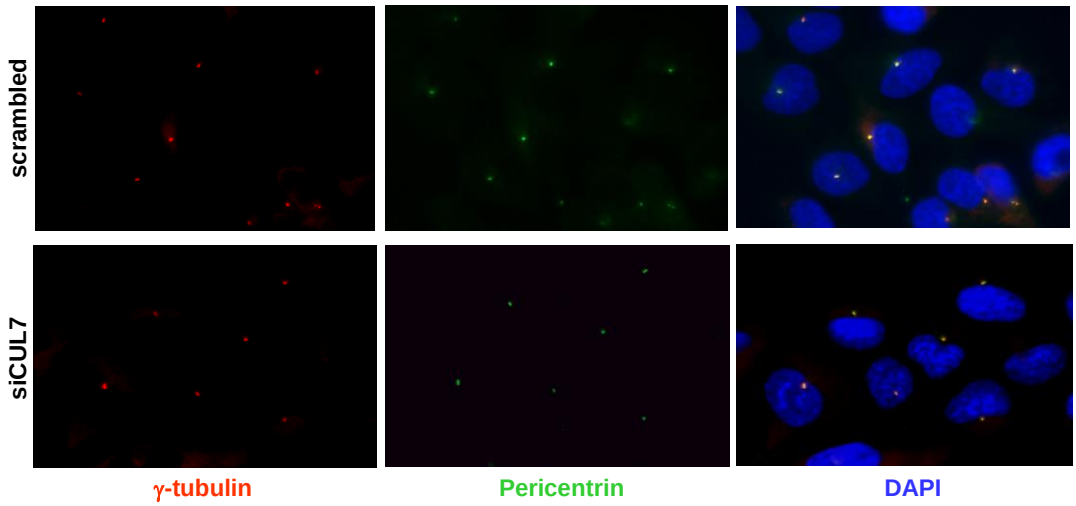

Figure S2. CUL7 depletion does not cause obvious MTOC defects, related to Figure 2.
U2OS cells were treated with scrambled or CUL7 siRNA. Three days after treatment, cells were fixed and stained with anti-γ-tubulin, anti-pericentrin, and DAPI. CUL7 depletion did not alter MTOC localization on the centrosome.

Yan et al. Supplementary Figure S3

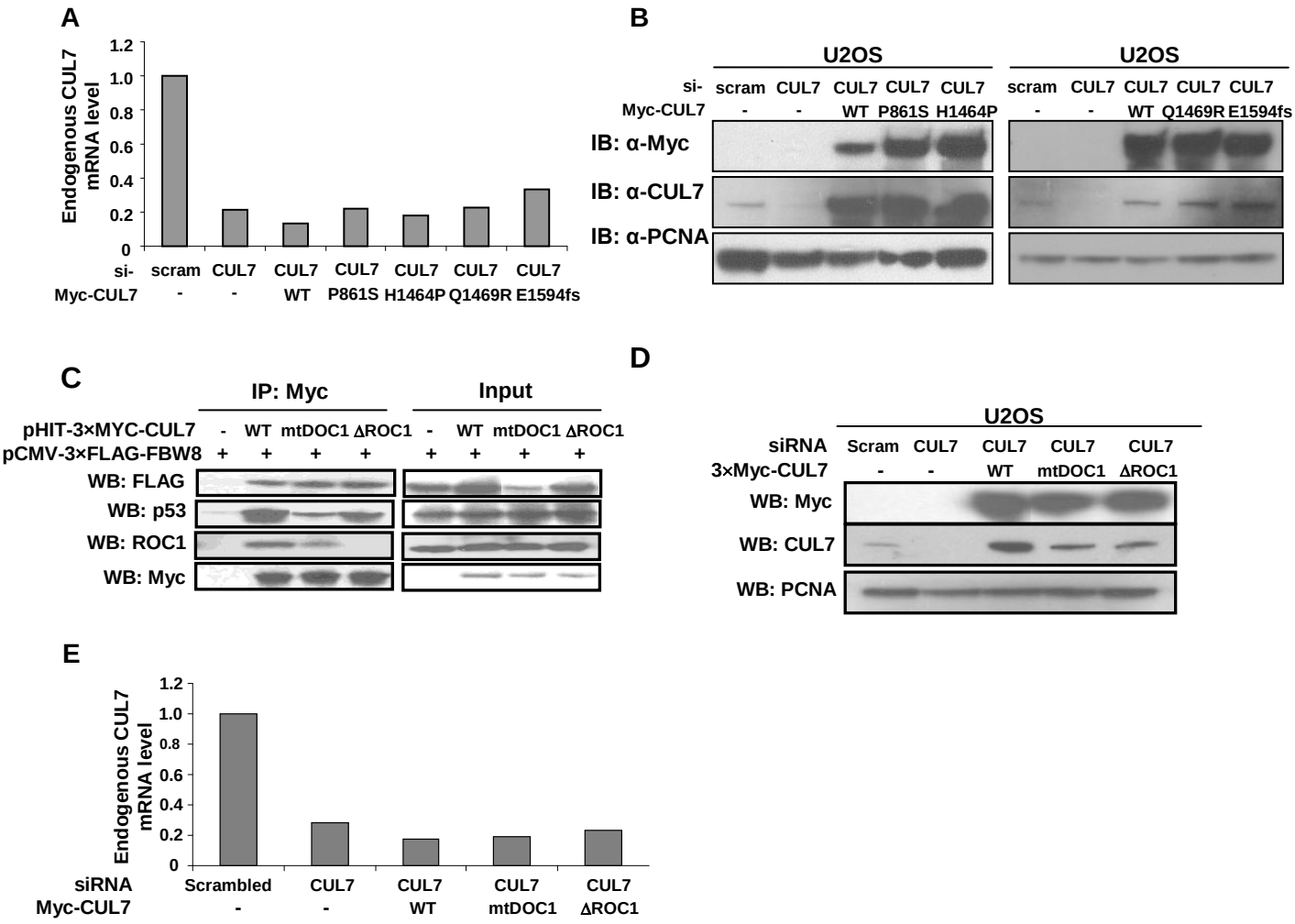

Figure S3. Wild type, but not 3-M associated mutants of CUL7, is able to rescue CUL7 depletion induced defects, related to Figure 3. (A, B) U2OS cells were transduced with retrovirus encoding siRNA resistant wild type and 3-M associated mutant CUL7, followed by the transfection with either scrambled or siCUL7 oligos. The efficiency of endogenous CUL7 and the rescue of CUL7 expression were tested by Q-RT-PCR or western blot. (C), 293T cells were co-transfected with pCMV-3×FLAG-FBXW8 and wild type pHIT-3×Myc-CUL7, or the indicated mutants. Cell lysates were incubated with anti-Myc antibody to pull-down Myc-CUL7 associated complexes. Immunoprecipitated proteins were then analyzed by western blot with the indicated antibodies. (D, E) U2OS cells were transduced with retrovirus encoding siRNA resistant wild type or indicated CUL7 mutants, followed by transfection of the indicated siRNA oligos. RNA (E) or protein (D) samples were then collected to determine the expression of CUL7 protein by western or endogenous mRNA levels by Q-RT-PCR. Q-RT-PCR was performed with primers targeting CUL7 mRNA 5'-UTR to avoid interference of exogenously expressed CUL7.

Yan et al. Supplementary Figure S4

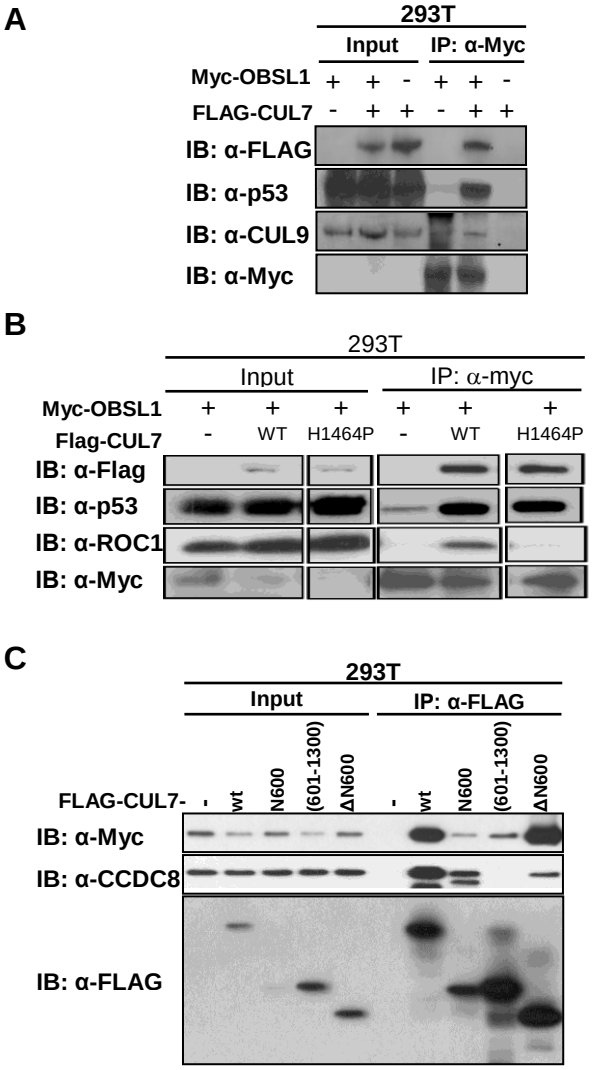

Figure S4. OBSL1 and CCDC8 associate with CUL7, related to Figure 4.

(**A, B, C**) 293T cells were transfected with plasmids expressing indicated proteins. Protein-protein interactions were determined by IP-western assays.

Yan et al. Supplementary Figure S5

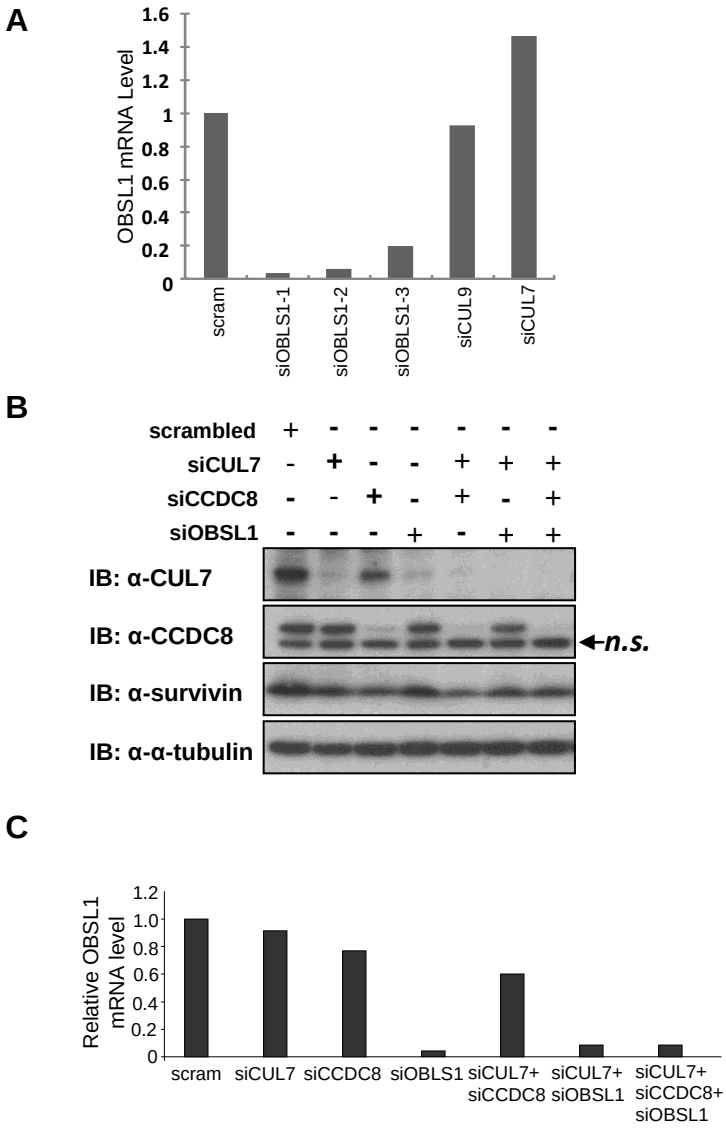

Figure S5. OBSL1 and CCDC8 regulate microtubule dynamics and mitosis, related to Figure 5.
(**A**) U2OS cells were treated with siRNA oligonucleotides against the indicated genes. Knocking down efficiency was determined by qRT-PCR.
(B, C) U2OS/EGFP-α-tubulin cells were transfected with the indicated siRNA oligos. Knocking down efficiency was determined by western blotting for CUL7 and CCDC8 **(B)** or by qRT-PCR for OBSL1 **(C)**.

Yan et al. Supplementary Figure S6

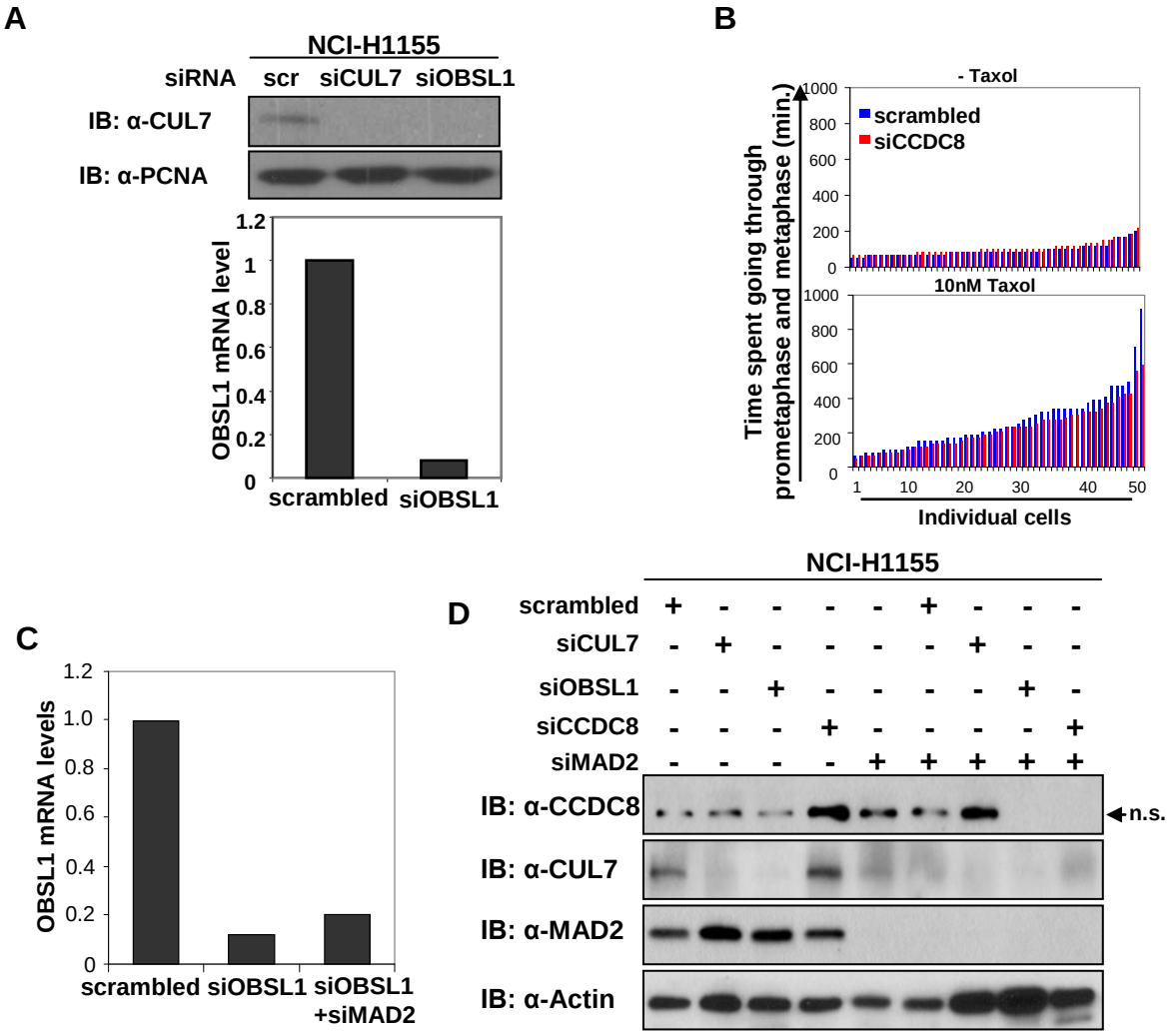

Figure S6. Loss of function of 3M genes sensitizes cells to microtubule damages, related to Figure 6.

(**A**) NCI-H1155/EGFP-H2B cells were transfected with indicated siRNA oligonucleotides. The expression of various proteins were determined by western blot. OBSL1 knock down efficiency was confirmed by Q-PCR. (**B**) Human lung cancer cells NCI-H1155 stably expressing EGFP-Histone H2B were transfected with scrambled or siCCDC8 siRNA oligos for 48 hours. The cells were then either untreated or treated with 10 nM paclitaxel for 24 hours, followed by imaging EGFP-Histone H2B every 14.5 minutes for 46 hours to trace mitosis. Duration from nuclear envelope break to anaphase was measured for 50 cells from each sample, and time intervals plotted. (**C** and **D**) CUL7 and OBSL1 depletion induced mitotic defects are SAC dependent, but CCDC8 is absent in NCI-H1155 cells. Note that the band detected in the blot is a non-specific (n.s.) cross reactive protein. NCI-H1155 cells stably expressing EGFP-Histone H2B were transfected with the indicated siRNA oligos for 48 hours. The knockdown efficiency of different siRNA oligos were confirmed with western **(D)** or Q-PCR **(C)**.

Yan et al. Supplementary Figure S7

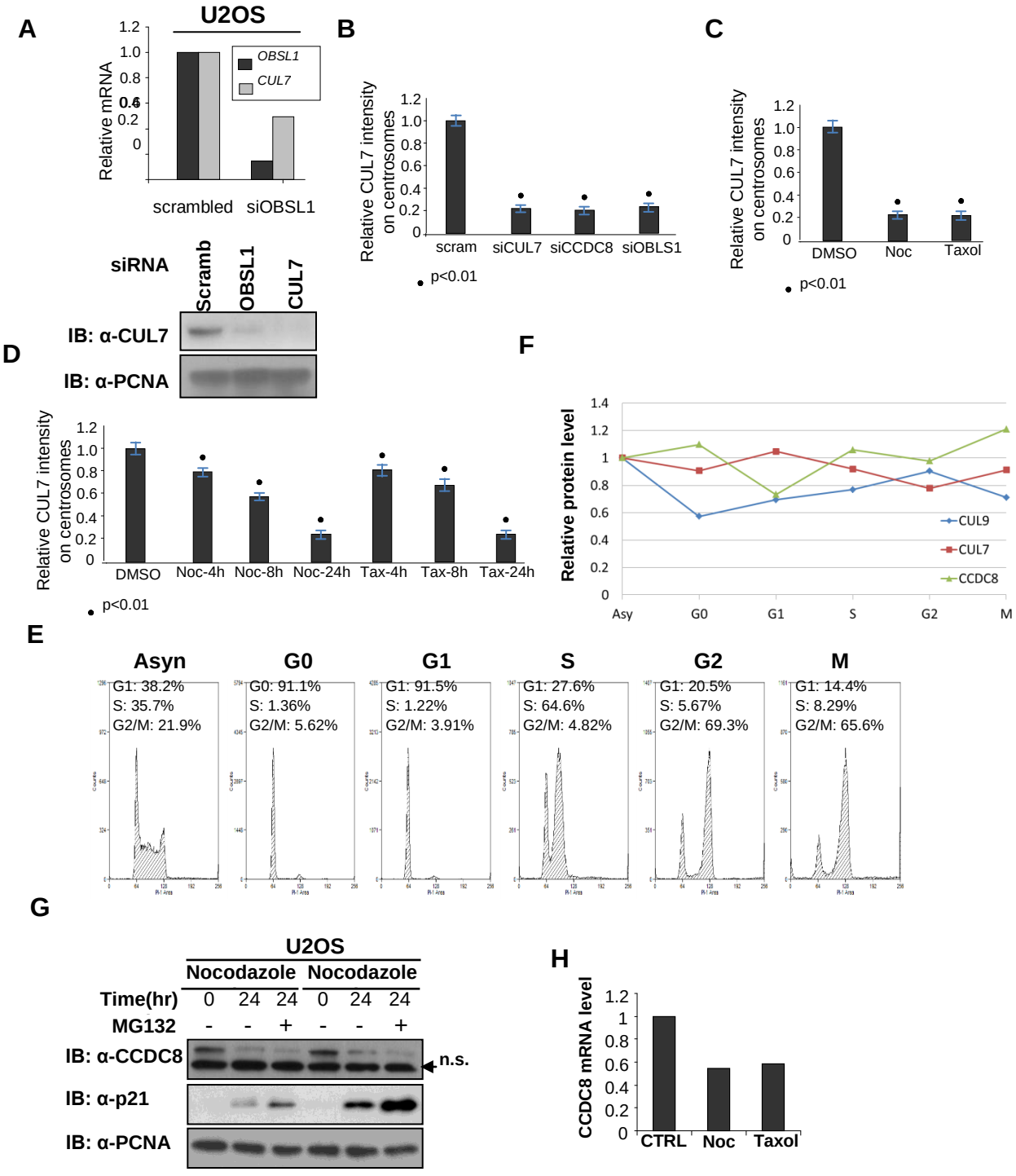

Figure S7. OBSL1 and CCDC8 regulate CUL7, related to Figure 7.

(**A**) U2OS cells were transfected with indicated siRNA oligonucleotides. The expression of various proteins and mRNA were determined by western blot and Q-PCR.

(**B**-**D**) Relative CUL7 intensity on centrosomes in Figure 7A (B), 7C (C), and 7E (D) was measured and plotted. Error bars indicate standard deviation. P values indicate significant differences to control cells by t-test.
(**E**) NHF1 cells were pre-synchronized at G0 phase by keeping them growing over-confluently for 2 days. Then cells were released to re-enter cell cycle by re-plating at low density. G1 cells were collected cells 8 hours after re-plating. To enrich the S- and G2 phase cells, contacted-inhibited cells were released into a media containing aphidicolin (2 µg/ ml) and cultured for 24 hours, followed by the switching into a fresh (drug-free) media for 4 or 8 hours before harvest to collect S- and G2 cells respectively. To enrich M-phase cells, 8 hours after switching cells from aphidicolin media into fresh media, colcemid (100 ng / ml) was added and cells were collected 4 hours later. The synchrony of each population was determined by FACS analysis, and western blotting of known cell cycle oscillating proteins was shown in Figure 7F.
(**F**) The relative levels of CUL7, CUL9, and CCDC9 in main Figure 7F were quantified and plotted.
(**G**) U2OS cells were treated with nocodazole or taxol in combination with MG132, and then the indicated proteins were determined by western blot.
(**H**) U2OS cells were treated with nocodazole or taxol and CCDC8 mRNA was determined by Q-PCR