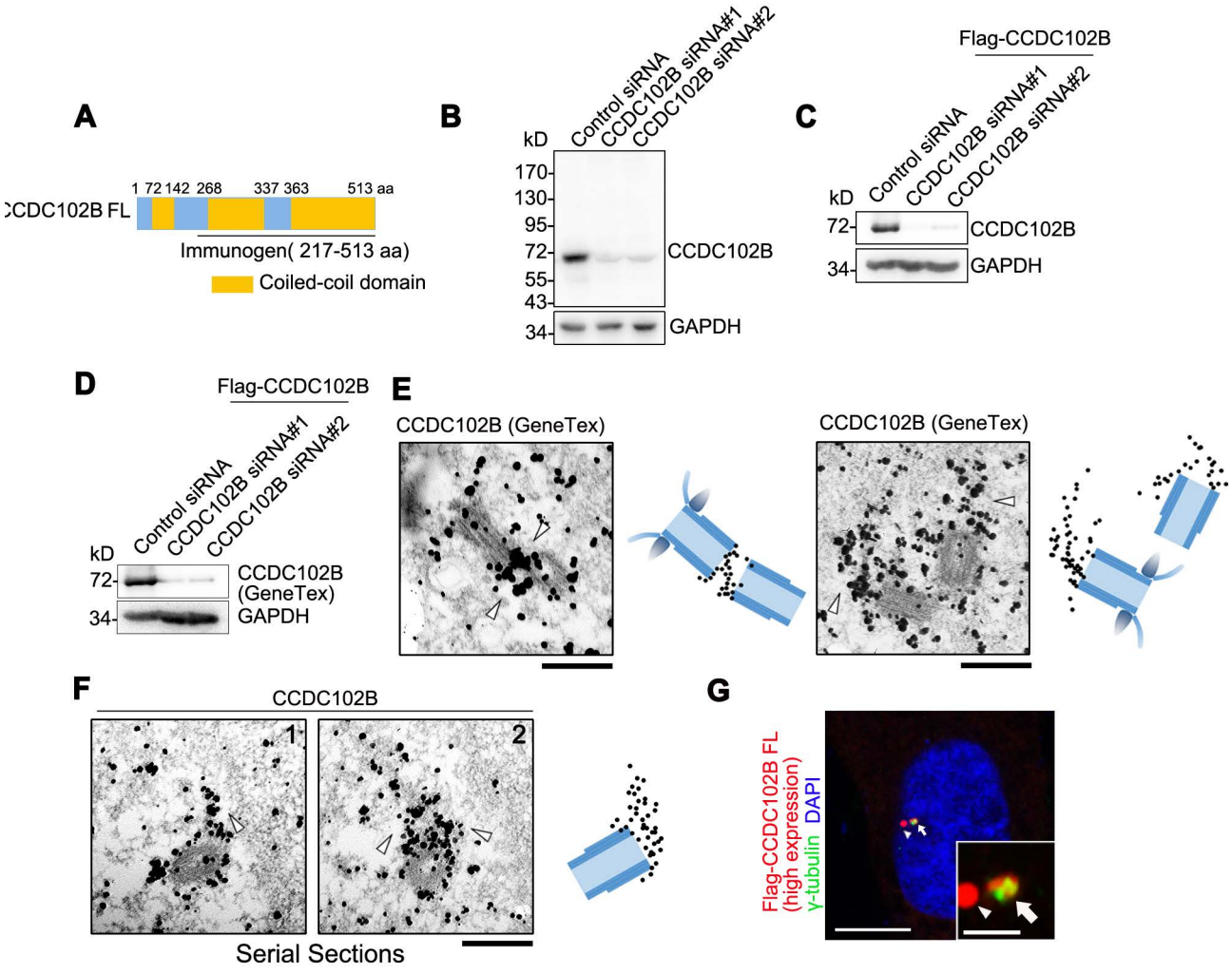


Figure S1. The specificity of CCDC102B antibodies and the localization of CCDC102B. (A) Schematic of CCDC102B. FL, full-length; coiled-coil domains, yellow. Immunogen, 217-513 aa of CCDC102B. (B) Lysates of HEK293T cells transfected with control- or CCDC102B-siRNA were subjected to immunoblotting using a lab-raised antibody against CCDC102B. GAPDH was used as a loading control. (C and D) After the treatment of control- or CCDC102B-siRNA for 24 h, U2OS cells were transfected with Flag-CCDC102B for an additional 48 h. Then the cell lysates were subjected to immunoblotting using lab-raised (C) or GeneTex (D) antibody against CCDC102B. GAPDH was used as a loading control. (E and F) Immuno-electron microscopy images. U2OS cells were labeled with GeneTex (E) and lab-raised (F) anti-CCDC102B antibodies, followed by nanogold-coupled secondary antibodies. A schematic of immuno-electron microscopy images is also shown. Arrowheads, CCDC102B. Scale bar: 500 nm. (G) Immunostaining of overexpressed Flag-tagged CCDC102B FL (red) and γ-tubulin (green) in U2OS cells. DNA was stained with 4,6-diamidino-2-phenylindole (DAPI, blue). Scale bars: 10 μm for complete images, and 2 μm in zoom. Arrow, centrosome. Arrowhead, Flag-CCDC102B aggregates.

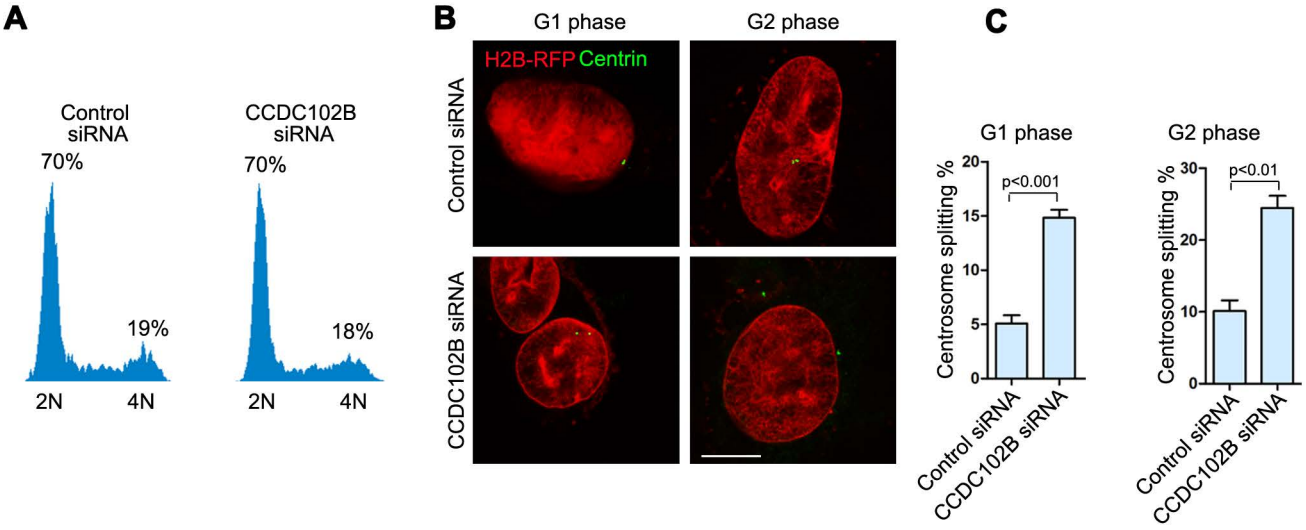


Figure S2. CCDC102B depletion induces centrosome splitting in the G1 and G2 phases. (A) U2OS cells transfected with control- or CCDC102B-siRNA were subjected to flow cytometry analysis. (B) Immunostaining of Centrin (green) in H2B-RFP- (red) overexpressed U2OS cells co-transfected with control- or CCDC102B-siRNA. The cells were synchronized to G1 and G2 phases. For synchronization in G1 phase, the cells were arrested in G2/M phase by nocodazole treatment for 24 h and then released for 6 h. For synchronization in G2 phase, the cells were arrested in G1/S phase by double-thymidine treatment and then released for 10-11 h. Scale bar: 10 μm. (C) Quantification of CCDC102B-depleted cells with centrosome splitting from (B). The data are presented as the mean±s.e.m. for three individual experiments with >50 cells per experiment. P-values are as indicated (two-tailed Student's *t*-test).

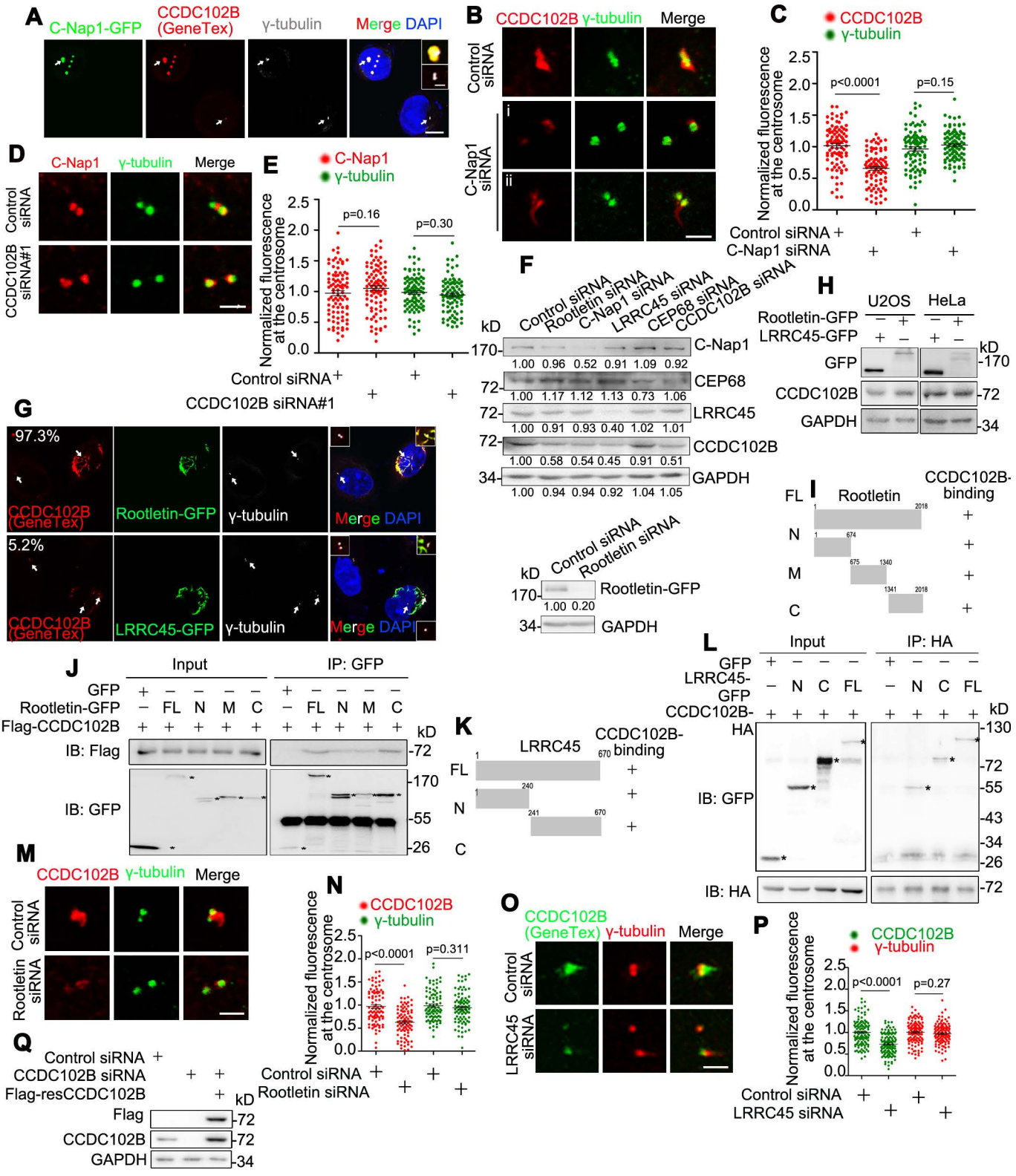


Figure S3. CCDC102B is recruited to the centrosome by C-Nap1, Rootletin and LRRC45. (A) Immunostaining of CCDC102B (red, GeneTex antibody) and γ-tubulin (white) in HeLa cells transfected with C-Nap1-GFP (green). DNA was stained with 4,6-diamidino-2-phenylindole (DAPI, blue). Arrow, centrosome. Scale bars: 10 μm for complete images, and 2 μm in zoom. (B) Immunostaining of CCDC102B (red, lab-raised antibody) and γ-tubulin (green) in U2OS cells transfected with control- or C-Nap1-siRNA. **i**, diminished centrosome localization of CCDC102B. **ii**, emanated long fibers of CCDC102B. Scale bar: 2 μm. (C) Quantitative analysis of the fluorescence intensity of CCDC102B and γ-tubulin at the centrosome from (B). Each dot represents a single cell. The data were pooled from three individual experiments, > 40 cells per experiment. The data are presented as the mean ± s.e.m; p-values are as indicated (two-tailed Student's *t*-test). (D) Immunostaining of C-Nap1 (red) and γ-tubulin (green) in U2OS cells transfected with control- or CCDC102B-siRNA. Scale bar: 2 μm. (E) Quantitative analysis of the fluorescence intensity of C-Nap1 and γ-tubulin at the centrosome from (D). Each dot represents a single cell. The data were pooled from three individual experiments, > 40 cells per experiment. The data are presented as the mean ± s.e.m; p-values are as indicated (two-tailed Student's *t*-test). (F) Immunoblots of lysates of U2OS cells transfected with control-, Rootletin-, C-Nap1-, LRRC45-, Cep68- or CCDC102B-siRNA, respectively. The antibodies used are as indicated. GAPDH was used as a loading control. Relative amounts of proteins were quantified and normalized to GAPDH. Three individual experiments were performed. (G) Immunostaining of CCDC102B (red, GeneTex antibody) and γ-tubulin (white) in HeLa cells transfected with GFP-tagged Rootletin (green) or LRRC45 (green). DNA was stained with DAPI (blue). Arrow, centrosome. Scale bars: 10 μm for complete images, and 2 μm in zoom. (H) Lysates of U2OS or HeLa cells transfected with Rootletin-GFP or LRRC45-GFP were subjected to immunoblotting. GAPDH was used as a loading control. (I)

Schematic of Rootletin full-length (FL) and its truncated mutants (N, M, C). +, positive. (J) Lysates of HEK293T cells overexpressing Flag-CCDC102B and the indicated GFP-tagged FL or truncated mutants of Rootletin were subjected to immunoprecipitation (IP) and immunoblotting (IB). *, indicates expected bands. (K) Schematic of LRRC45 FL and its truncated mutants (N, C). +, positive. (L) Lysates of HEK293T cells overexpressing CCDC102B-HA and the indicated GFP-tagged FL or truncated mutants of LRRC45 were subjected to IP and IB. *, indicates expected bands. (M) Immunostaining of CCDC102B (red) (lab-raised antibody) and γ-tubulin (green) in U2OS cells transfected with control- or Rootletin-siRNA. Scale bar: 2 μm. (N) Quantitative analysis of the fluorescence intensity of CCDC102B and γ-tubulin at the centrosome from (M). Each dot represents a single cell. The data were pooled from three individual experiments, > 40 cells per experiment. The data are presented as the mean ± s.e.m; p-values are as indicated (two-tailed Student's *t*-test). (O) Immunostaining of CCDC102B (green, GeneTex antibody) and γ-tubulin (red) in U2OS cells transfected with control- or LRRC45-siRNA. Scale bar: 2 μm. (P) Quantitative analysis of the fluorescence intensity of CCDC102B and γ-tubulin at the centrosome from (O). Each dot represents a single cell. The data were pooled from three individual experiments, > 40 cells per experiment. The data are presented as the mean ± s.e.m; p-values are as indicated (two-tailed Student's *t*-test). (Q) Immunoblots of CCDC102B (lab-raised antibody) in U2OS cells transfected with control- or CCDC102B-siRNA and rescued by exogenously expressing siRNA-resistant CCDC102B (Flag-resCCDC102B). GAPDH was used as a loading control.

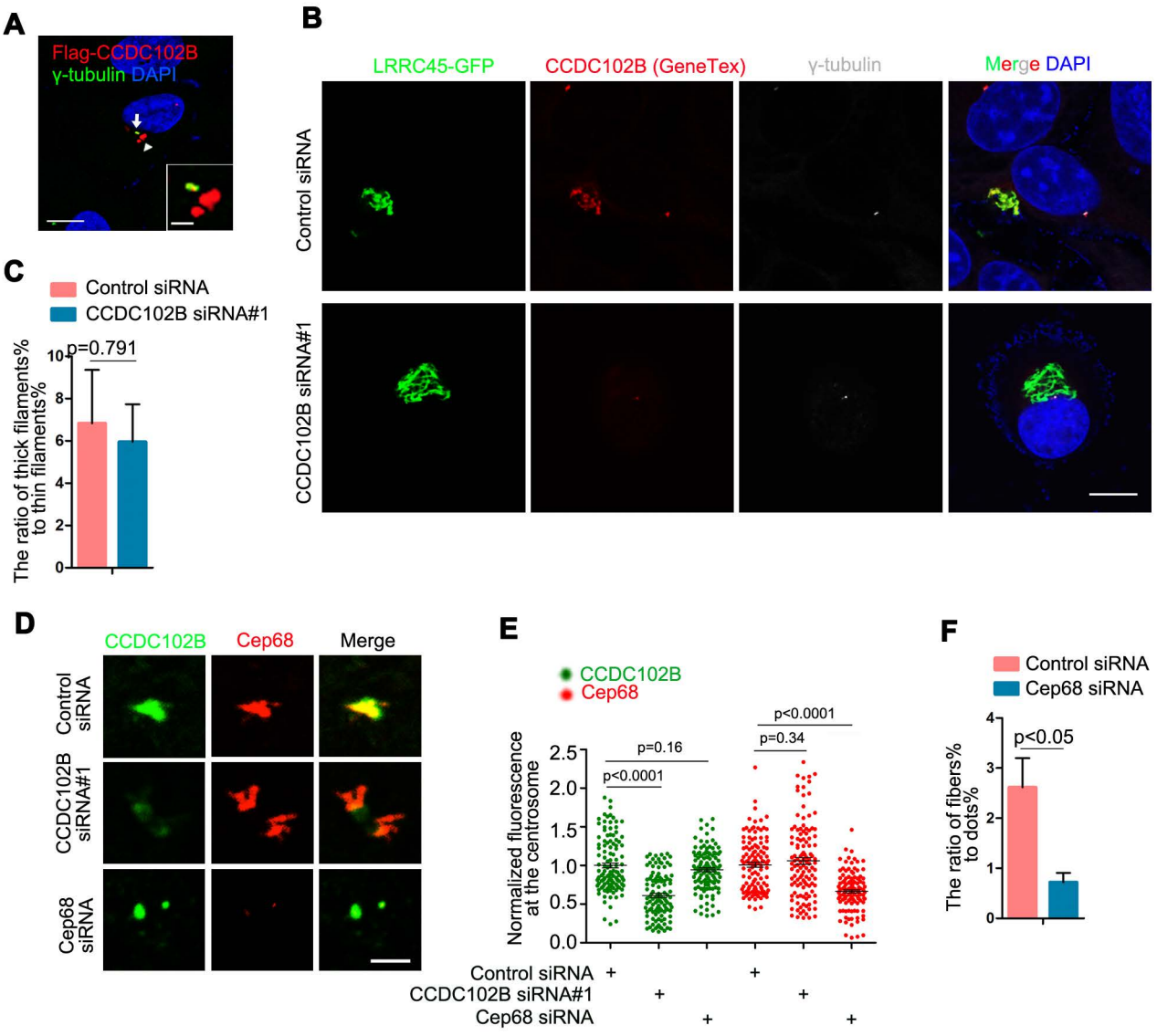
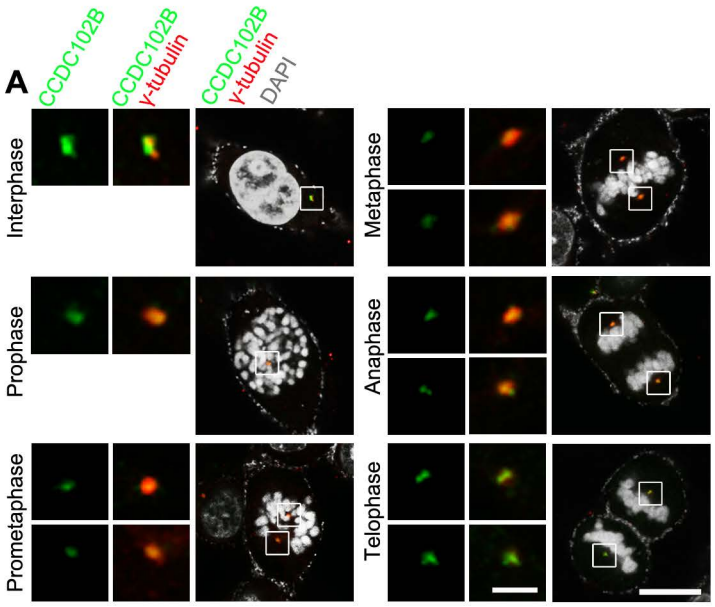
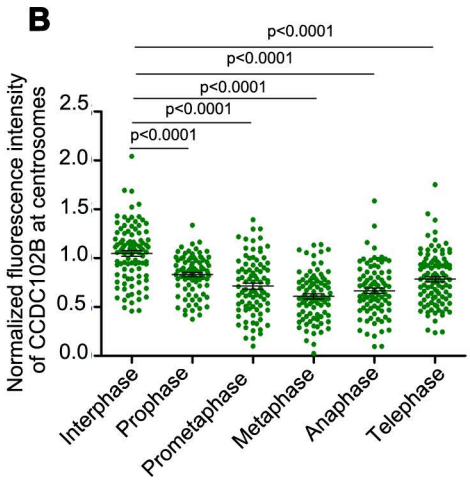


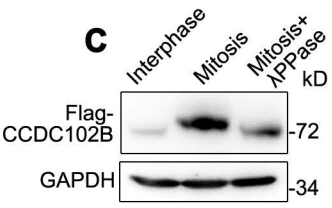
Figure S4. CCDC102B fibers are decreased after the loss of Cep68. (A) Immunostaining of overexpressed Flag-CCDC102B (red) and γ-tubulin (green) in U2OS cells. DNA was stained with 4,6-diamidino-2-phenylindole (DAPI, blue). Arrow, centrosome; arrowhead, Flag-CCDC102B aggregates. Scale bars, 10 μm for complete images, and 2 μm for magnified centrosomes. (B) Immunostaining of CCDC102B (red, GeneTex antibody) and γ-tubulin (white) in U2OS cells transfected LRRC45-GFP and control- or CCDC102B-siRNA. DNA was stained with DAPI (blue). Scale bar, 10 μm. After treatment with the indicated siRNAs for 24 h, LRRC45-GFP was transfected for an additional 48 h. (C) Quantitative analysis of the ratio of the percentage of cells with thick to that with thin LRRC45 filaments from (B). Three individual experiments, > 100 cells per experiment. The data are presented as the mean ± s.e.m; p-values are as indicated (two-tailed Student's *t*-test). (D) Immunostaining of CCDC102B (green, lab-raised antibody) and Cep68 (red) in U2OS cells transfected with control-, CCDC102B-, or Cep68-siRNA. Scale bar: 2 μm. (E) Quantitative analysis of the fluorescence intensity of CCDC102B and Cep68 at the centrosome from (D). Each dot represents a single cell. The data were pooled from three individual experiments, > 40 cells per experiment. The data are presented as the mean ± s.e.m; p-values are as indicated (one-way ANOVA). (F) Quantitative analysis of the ratio of the percentage of cells with CCDC102B showing the fiber-like to that showing dot-like structures from (D). Three individual experiments, > 100 cells per experiment. The data are presented as the mean ± s.e.m; p-values are as indicated (two-tailed Student's *t*-test).

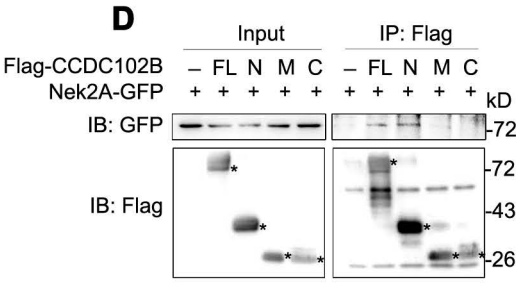


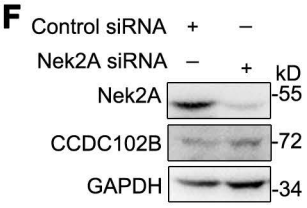


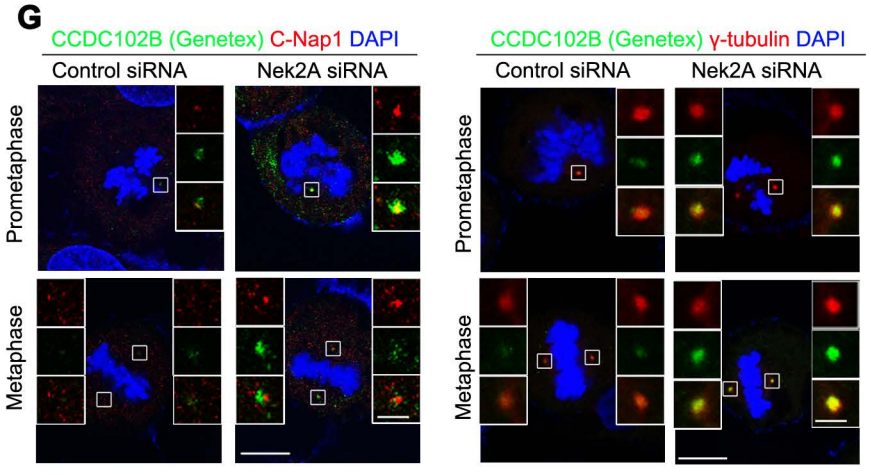
E

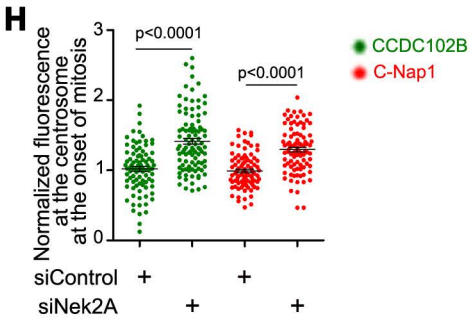
Sequence	Modifications
LIEETQIFQMQQsSIK	S21(Phospho)
LIEETQIFQMQQSsIK	S22(Phospho)
GDMVAPAsPPR	S34(Phospho)
ELsTLKK	S135(Phospho)
KKQsLPPQK	S142(Phospho)
RQFsTKEDTNNK	S194(Phospho)
EQGVVIDsLK	S210(Phospho)
KNRLsANSQSPDFK	S401(Phospho)
NRLsANsQSPDFK	S401(Phospho)
	S404(Phospho)
NRLsANsQsPDFK	S401(Phospho)
	S404(Phospho)
	S406(Phospho)











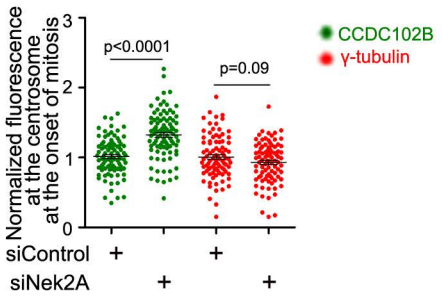


Figure S5. CCDC102B is phosphorylated by Nek2A. (A) Immunofluorescence analysis of CCDC102B (green, lab-raised antibody) and γ-tubulin (red) in HeLa cells. DNA was stained with 4,6-diamidino-2-phenylindole (DAPI, white). Scale bars: 10 μm for complete images, and 5 μm for magnified centrosomes. (B) Quantitative analysis of CCDC102B fluorescence intensity at the centrosome from (A). Each dot represents a single cell. The data were pooled from three individual experiments, > 40 cells per experiment. The data are presented as the mean ± s.e.m; p-values are as indicated (one-way ANOVA). (C) Mitotic HeLa cells transfected with Flag-CCDC102B were treated with λ-PPase and subjected to immunoblotting using phos-tag gels. The cells were arrested in prometaphase using nocodazole. GAPDH was used as a loading control. (D) Lysates of HEK293T cells overexpressing Nek2A-GFP and the indicated Flag-tagged full-length (FL) or truncated mutants (N, M, and C) of CCDC102B were subjected to immunoprecipitation (IP) and immunoblotting (IB). N, 1-216 aa; M, 217-349 aa; C, 350-513 aa. *, indicates expected bands. (E) Phosphorylation sites of CCDC102B identified by mass spectrometry. HEK293T cells transfected with Flag-CCDC102B and Nek2A-WT-GFP or Nek2A-K37R-GFP were subjected to immunoprecipitation and mass spectrometry analysis. WT, wild-type; K37R, kinase-dead mutant. (F) Lysates of U2OS cells transfected with control- or Nek2A-siRNA were subjected to immunoblotting with the indicated antibodies. GAPDH was used as a loading control. (G) Immunostaining of CCDC102B (green, GeneTex antibody) and C-Nap1 (red) or γ-tubulin (red) in synchronized U2OS cells after transfection with control- or Nek2A-siRNA. DNA was stained with DAPI (blue). Scale bars: 10 μm for complete images, and 2 μm for magnified centrosomes. (H) Quantification of the fluorescence intensities at the centrosome from (G). Each dot represents a single cell. The data were pooled from three individual experiments, > 40 cells per experiment. The data are presented as the mean ± s.e.m; p-values are as indicated (two-tailed Student's *t*-test).