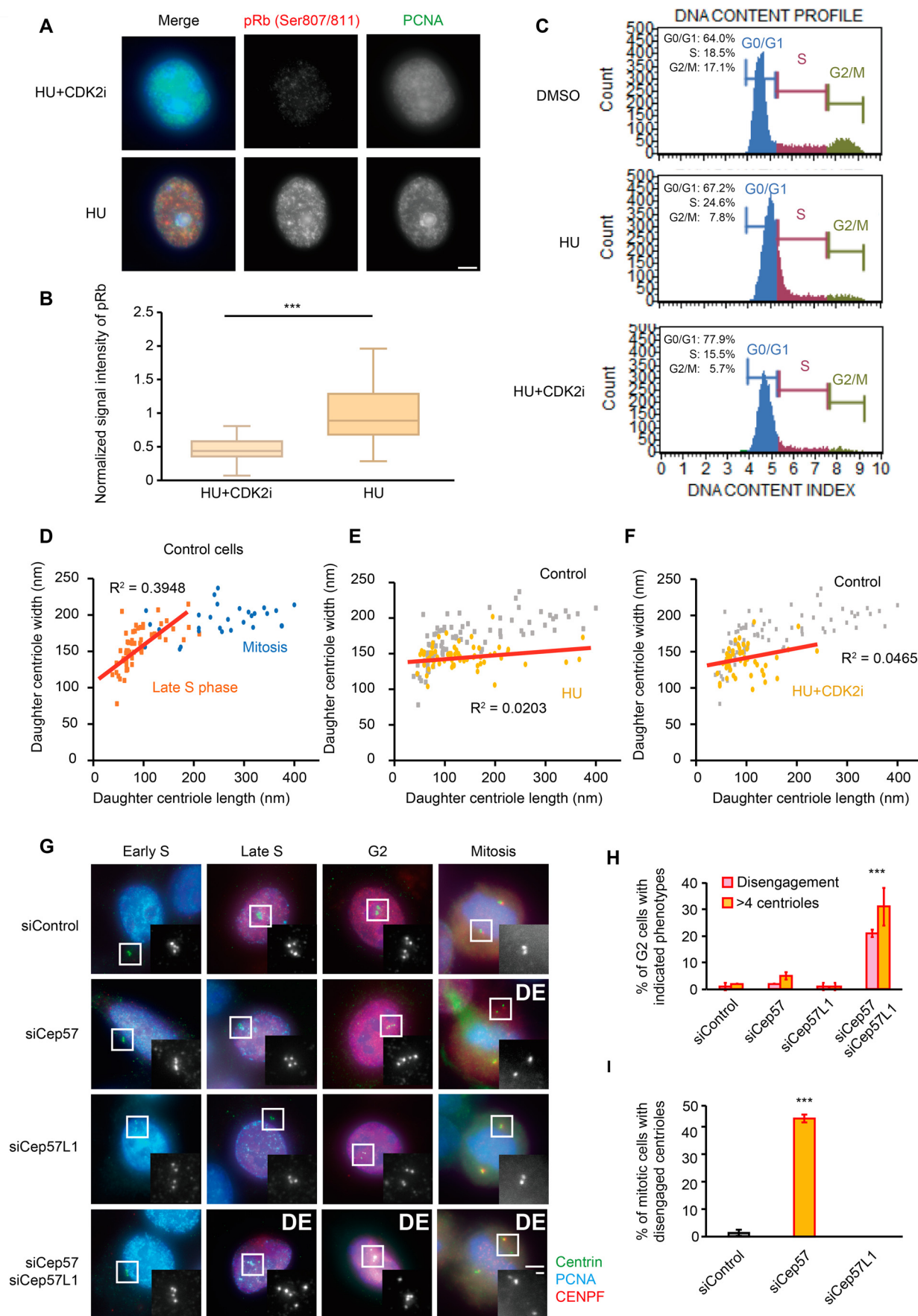
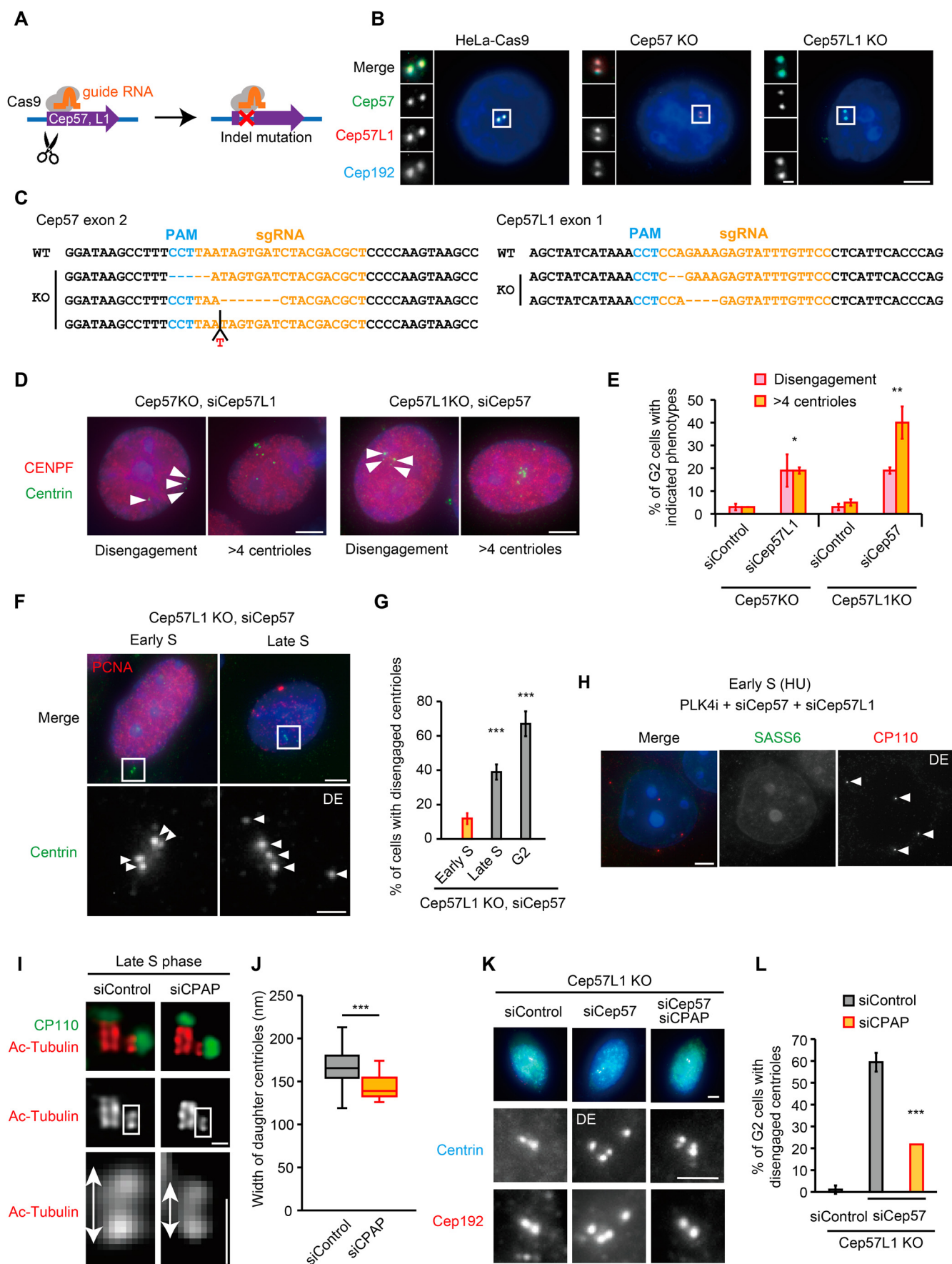


Expanded View Figures

Figure EV1. Effect of HU and CDK2 inhibitor treatments on centriole and cell cycle.

(A) Representative immunofluorescence images of HeLa cells treated with HU or HU + CDK2 inhibitor. Scale bar: 5 μm . (B) Quantification of the p-Rb (Ser807/811) signal intensity in the nucleus from (A). Boxplot was employed to illustrate the data distribution. The central line represents the median, the box spans the interquartile range (IQR) from the 25th to the 75th percentile, and the whiskers extend to the smallest and largest data points within 1.5 times the IQR. *P* values were calculated by two-tailed unpaired Student's *t*-test ($P = 1.57\text{E}-08$). *** $P < 0.001$. (C) Quantification of the DNA content in HeLa cells treated as indicated, using flow cytometric analysis. (D-F) Scatter plot quantification of the length and the width of the daughter centrioles from Fig. 1. (G) Representative immunofluorescence images of HeLa cells in early S phase, late S phase, G2 phase, and mitosis. Scale bars: 5 μm , 1 μm . (H) Quantification of the frequency of cells with disengaged centrioles in G2 phase in (G). Error bars represent the mean $\pm$ s.d. of three biological independent experiments. *P* value was calculated by Dunnett's multiple comparisons test ($P = 1.24\text{E}-07$). *** $P < 0.001$. (I) Quantification of the frequency of cells with disengaged centrioles in mitosis in (G). Error bars represent the mean $\pm$ s.d. of three biological independent experiments. *P* value was calculated by Dunnett's multiple comparisons test ($P = 8.06\text{E}-10$). *** $P < 0.001$. Source data are available online for this figure.





◀ **Figure EV2. Confirmation of the centriole engagement by Cep57 and Cep57L1 in interphase using knockout cell lines.**

(A) Schematic illustration for generating knockout (KO) cells using CRISPR-Cas9. (B) Immunofluorescence images of HeLa-Cas9, Cep57KO, and Cep57L1KO cells. Scale bars: 5 μ m, 1 μ m. (C) DNA sequences surrounding the CRISPR-targeted regions in the exons of the Cep57 and Cep57L1 genes. (D) Representative immunofluorescence images of HeLa-Cep57KO cells transfected with siCep57L1, and Cep57L1KO cells transfected with siCep57 in G2 phase. Scale bars: 5 μ m. White arrowheads indicate the centrioles. (E) Quantification of the frequency of cells possessing four disengaged centrioles and more than four centrioles in G2 phase in (D). Error bars represent the mean $\pm$ s.d. of three biological independent experiments. *P* value was calculated by two-tailed unpaired Welch's t-test with Bonferroni correction (Cep57KO, *P* = 0.0230; Cep57L1KO, *P* = 0.00146). **P* < 0.05; ***P* < 0.01. (F) Representative immunofluorescence images of HeLa-Cep57L1KO cells transfected with siCep57 in early and late S phase. Scale bars: 5 μ m, 1 μ m. White arrowheads indicate the centrioles. (G) Quantification of the frequency of the cells with disengaged centrioles in early S, late S, and G2 phase in (F). Error bars represent the mean $\pm$ s.d. of three biological independent experiments. *P* values were calculated by Dunnett's multiple comparisons test (Late S, *P* = 0.000192; G2, *P* = 1.70E-06). ****P* < 0.001. (H) Representative immunofluorescence images of HeLa cells subjected to a cartwheel removal assay. HeLa cells were treated with HU and CDK2 inhibitor III (CDK2i) for 24 h, followed by treatment with a PLK4 inhibitor for another 24 h. Scale bar: 5 μ m. White arrowheads indicate the centrioles. (I) Representative STED microscopy images of HeLa cells transfected with siControl or siCPAP. Double-headed arrows indicate the width of centrioles. Scale bars: 200 nm. (J) Quantification of the width of the daughter centrioles in (I). *n* = 40 centrioles pooled from 3 independent experiments. *P* value was calculated by two-tailed unpaired Welch's t-test (*P* = 5.54E-07). ****P* < 0.001. (K) Representative immunofluorescence images of HeLa Cep57L1 KO cells transfected with siControl, siCep57, or siCep57 + siCPAP. Scale bars: 5 μ m, 1 μ m. (L) Quantification of the frequency of cells with disengaged centrioles in (K). Error bars represent the mean $\pm$ s.d. of three biological independent experiments. *P* value was calculated by two-tailed unpaired Welch's t-test (*P* = 0.000419). ****P* < 0.001. Source data are available online for this figure.

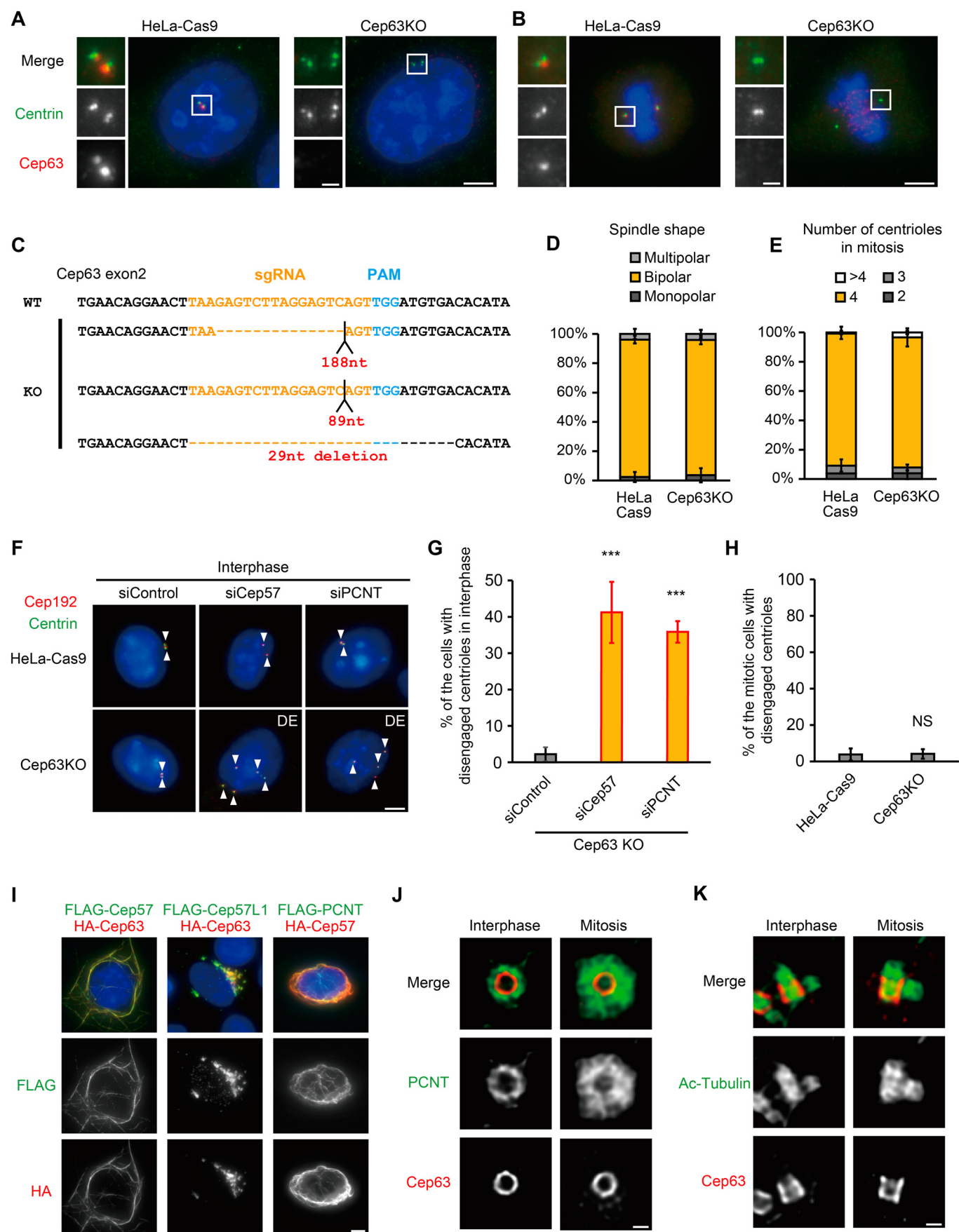
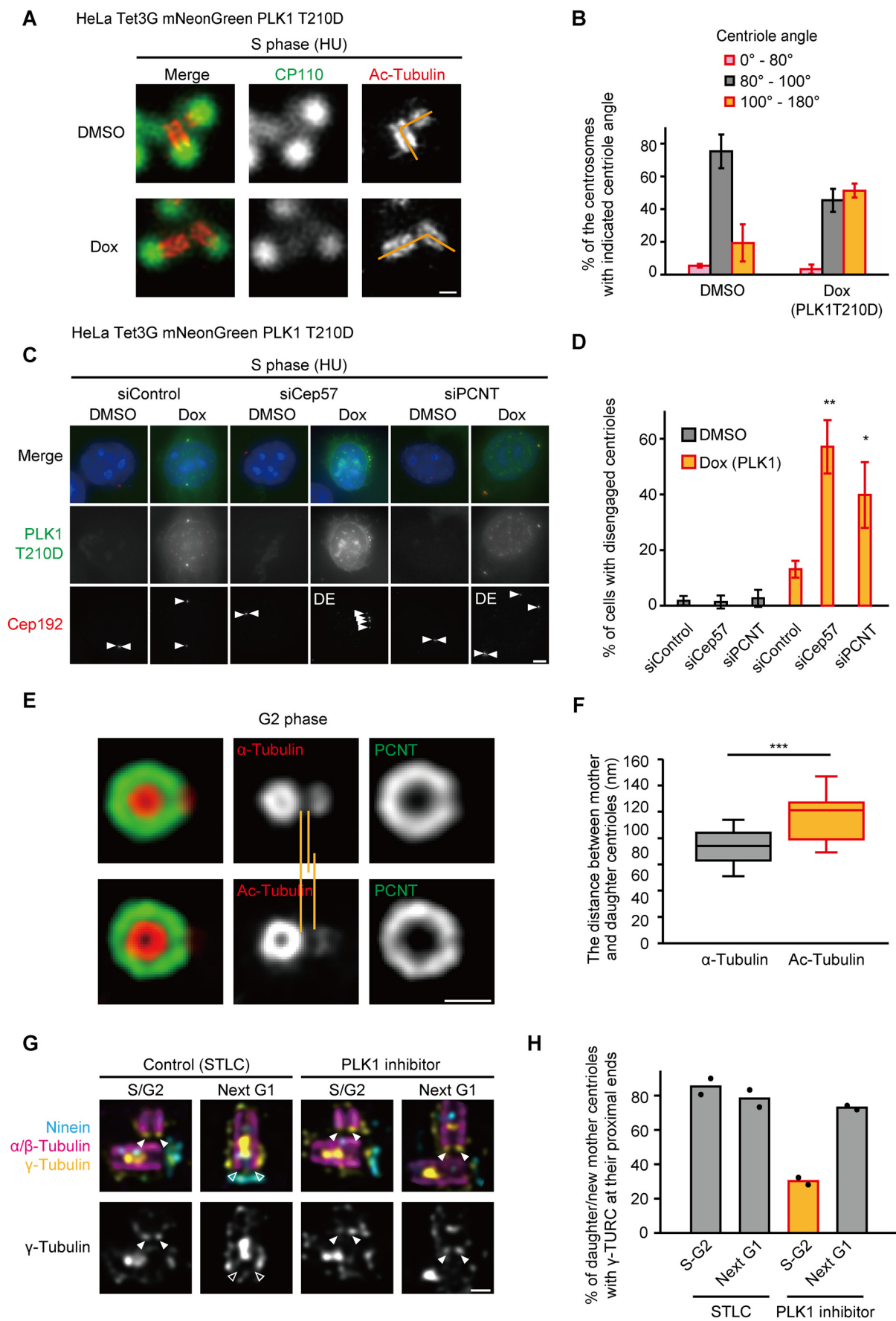


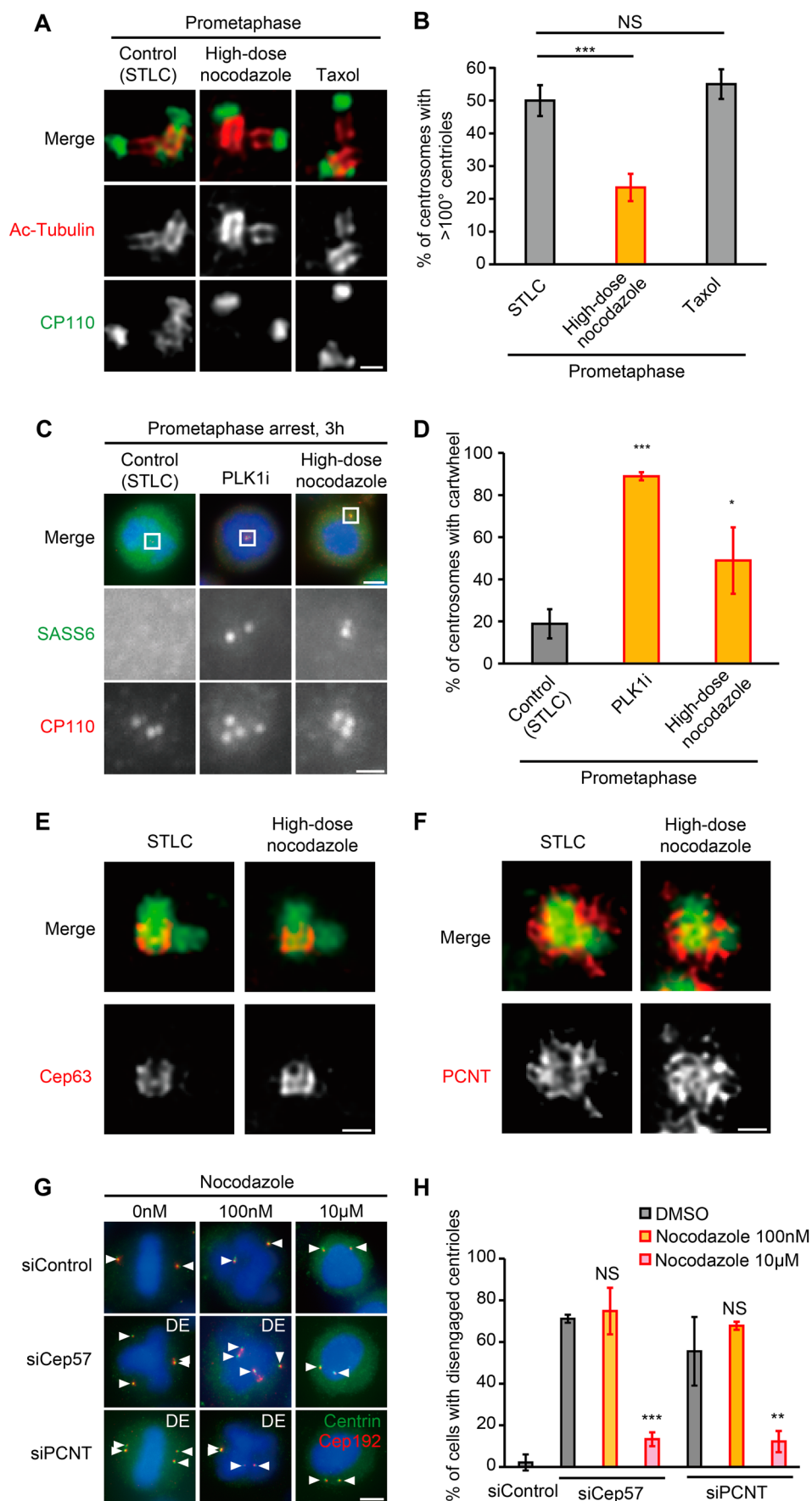

Figure EV3. Cep63 is involved in centriole engagement.

(A) Immunofluorescence images of HeLa-Cas9 and Cep63KO cells in interphase. Scale bars: 5 μ m, 1 μ m. (B) Immunofluorescence images of HeLa-Cas9 and Cep63KO cells in mitosis. Scale bars: 5 μ m, 1 μ m. (C) DNA sequences surrounding the CRISPR-targeted regions in the exons of the *Cep63* gene. (D) Quantification of the frequency of mitotic cells with the indicated spindle shape. Error bars represent the mean $\pm$ s.d. of three biological independent experiments. (E) Quantification of the frequency of mitotic cells with the indicated number of centrioles in (B). Error bars represent the mean $\pm$ s.d. of three biological independent experiments. (F) Representative immunofluorescence images of HeLa-Cas9 and Cep63KO cells transfected with siControl, siCep57 or siPCNT in interphase. Scale bar: 5 μ m. White arrowheads indicate the centrioles. (G) Quantification of the frequency of cells with disengaged centrioles in (F). Error bars represent the mean $\pm$ s.d. of three biological independent experiments. *P* values were calculated by Dunnett's multiple comparisons test (siCep57: *P* = 0.000234, siPCNT: *P* = 0.000496). ****P* < 0.001. (H) Quantification of the frequency of mitotic cells with disengaged centrioles. Error bars represent the mean $\pm$ s.d. of three biological independent experiments. *P* value was calculated by two-tailed unpaired Welch's *t*-test (*P* = 0.912). NS, not significant. (I) Immunofluorescence images of HeLa cells overexpressing Cep57, Cep57L1, Cep63, or PCNT after plasmid transfection. Scale bar: 5 μ m. (J) STED microscopy image showing the top view of a mother centriole in G2 and mitosis. Scale bar: 200 nm. (K) STED microscopy image showing the side view of a mother centriole in G2 and mitosis. Scale bar: 200 nm. Source data are available online for this figure.



◀ **Figure EV4. PLK1 overexpression changes the configuration and the mechanism of centriole engagement.**

(A) Representative STED microscopy images of HeLa-tet3G-PLK1T210D cells treated with DMSO or Doxycycline in HU-arrested S phase. Scale bar: 200 nm. (B) Quantification of the frequency of cells with indicated centriole angle in (A). Error bars represent the mean $\pm$ s.d. of three biological independent experiments. (C) Representative immunofluorescence images of HeLa-tet3G-PLK1T210D cells treated with DMSO or Doxycycline in HU-arrested S phase. Scale bar: 5 μ m. White arrowheads indicate the centrioles. (D) Quantification of the frequency of cells with disengaged centrioles in (C). Error bars represent the mean $\pm$ s.d. of three biological independent experiments. *P* values were calculated by Dunnett's multiple comparisons test (HU + Dox + siCep57: *P* = 0.00490, siPCNT: *P* = 0.0447). **P* < 0.05; ***P* < 0.01. (E) Representative STED microscopy images of centrioles in G2 phase immunostained with anti-PCNT and anti- α -tubulin or anti-acetylated tubulin antibodies. Scale bar: 200 nm. (F) Quantification of the distance between the mother centriole wall and the proximal end of the daughter centriole in (E). Boxplot was employed to illustrate the data distribution. The central line represents the median, the box spans the interquartile range (IQR) from the 25th to the 75th percentile, and the whiskers extend to the smallest and largest data points within 1.5 times the IQR. *P* value was calculated by two-tailed unpaired Student's *t*-test (*P* = 0.000329). ****P* < 0.001. (G) Representative U-ExM images of daughter and new mother centrioles in HeLa cells treated with STLC or the PLK1 inhibitor BI2536. New mother centrioles were obtained from cells forced into G1 by treatment with the prometaphase-arresting drugs STLC or BI2536 for 3 h, followed by the addition of the CDK1 inhibitor RO3306 (10 μ M) for 3 h. New mother centrioles are identified by the absence of ODF2 signal at the distal part. Filled and open arrowheads indicate the proximal end of daughter/new mother centrioles with and without γ -tubulin signals, respectively. Scale bar: 200 nm. (H) Quantification of the frequency of the daughter or new mother centrioles with γ -tubulin signal at its proximal end in (G). Two biologically independent experiments, 30 cells each. Details of the quantification method are provided in the Methods section. Source data are available online for this figure.



◀ **Figure EV5. The proximal end of the daughter centriole is depolymerized in mitosis.**

(A) Representative STED microscopy images of centrioles in mitosis treated with DMSO, high-dose nocodazole (10 μ M), or taxol for 3 h. Scale bar: 200 nm. (B) Quantification of the frequency of the centrosomes, in which the centrioles are connected at an obtuse angle in (A). Error bars represent the mean $\pm$ s.d. of three biological independent experiments. *P* values were calculated by Dunnett's multiple comparisons test (Nocodazole: *P* = 0.000458, Taxol: *P* = 0.291). NS, not significant; ****P* < 0.001. (C) Representative immunofluorescence images of HeLa cells in mitosis treated with STLC, PLK1 inhibitor, or high-dose nocodazole for 3 h. Scale bar: 5 μ m, 1 μ m. (D) Quantification of the frequency of cells with cartwheel-retaining centrosomes in (C). Error bars represent the mean $\pm$ s.d. of three biological independent experiments. *P* values were calculated by Dunnett's multiple comparisons test (PLK1i: *P* = 0.00029, Nocodazole: *P* = 0.0185). **P* < 0.05; ****P* < 0.001. (E, F) Representative STED microscopy images of centrioles in HeLa cells. Scale bars: 200 nm. (G) Representative immunofluorescence images of HeLa cells in mitosis transfected with siControl, siCep57, or siPCNT, under treatment with different doses of nocodazole. Scale bar: 5 μ m. White arrowheads indicate the centrioles. (H) Quantification of the frequency of cells with disengaged centrioles in mitosis in (G). Error bars represent the mean $\pm$ s.d. of three biological independent experiments. *P* values were calculated by Dunnett's multiple comparisons test (siCep57 100 nM nocodazole: *P* = 0.739, 10 μ M nocodazole: *P* = 0.000163; siPCNT 100 nM nocodazole: *P* = 0.299, 10 μ M nocodazole: *P* = 0.00297). NS, not significant; ***P* < 0.01; ****P* < 0.001. Source data are available online for this figure.