



**Figure S4** Cep57 is required for proper spindle microtubule organization. **(A)** Immunoblot of Cep57 in control and Cep57 siRNA-transfected CHO cells at 72 h post-transfection. GAPDH expression levels were used as controls. **(B)** CHO cells were transfected with pSuper-Cep57-RFP expressing RFP, which allowed us to identify the Cep57-depleted cells. The cells were fixed and immunostained for Cep57. The cell with RFP contained no Cep57

centrosome signal. Bar, 5  $\mu\text{m}$ . **(C)** Cells in metaphase were subjected to immunoelectron microscopy. Cells were labeled with anti-Cep57 antibody followed by a nanogold-coupled secondary antibody. Gold particles can be seen close to the microtubules (arrowheads). The inset shows a higher magnification image of the cell. Bar, 100 nm. **(D and E)** Exogenous Cep57 can stabilize microtubules. **(D)** CHO cells were transfected with Cep57-GFP and treated with 5  $\mu\text{g/ml}$  nocodazole for 5 min. The cells were immunostained with anti- $\alpha$ -tubulin antibody (red). Representative cells with different Cep57 exogenous expression levels. Note that Cep57 at a low expression level (centrosome, no bundled microtubules could be detected) can also stabilize microtubules to resist nocodazole treatment. Bar, 5  $\mu\text{m}$ . **(E)** CHO cells expressing GFP (Control), Cep57 (1-242)-GFP (Cep57 N-GFP, without microtubule binding domain), and Cep57-full-length-GFP (Cep57 FL-GFP) were treated with nocodazole for 30 min and then fixed and labeled with anti- $\alpha$ -tubulin antibody. The microtubules showed three phenotypes: no intact (brown), limited (white) and extensive (grey) microtubules. Bar, 5  $\mu\text{m}$ . The histogram showed quantification of the percentage of cells with different microtubule phenotypes. Note that the full length of Cep57 (Cep57 FL-GFP) can stabilize microtubules. GFP and Cep57 N-GFP were used as negative controls. A minimum of 200 cells was counted per sample in three independent experiments. Error bars represent mean  $\pm$  SEM. \*\*  $P < 0.001$ , unpaired Student's  $t$ -test. **(F)** Electron microscopy images of the representative control spindles and Cep57 RNAi spindles. Insets show high magnification images of the spindle poles. Bars, 1  $\mu\text{m}$ .