

Expanded View Figures

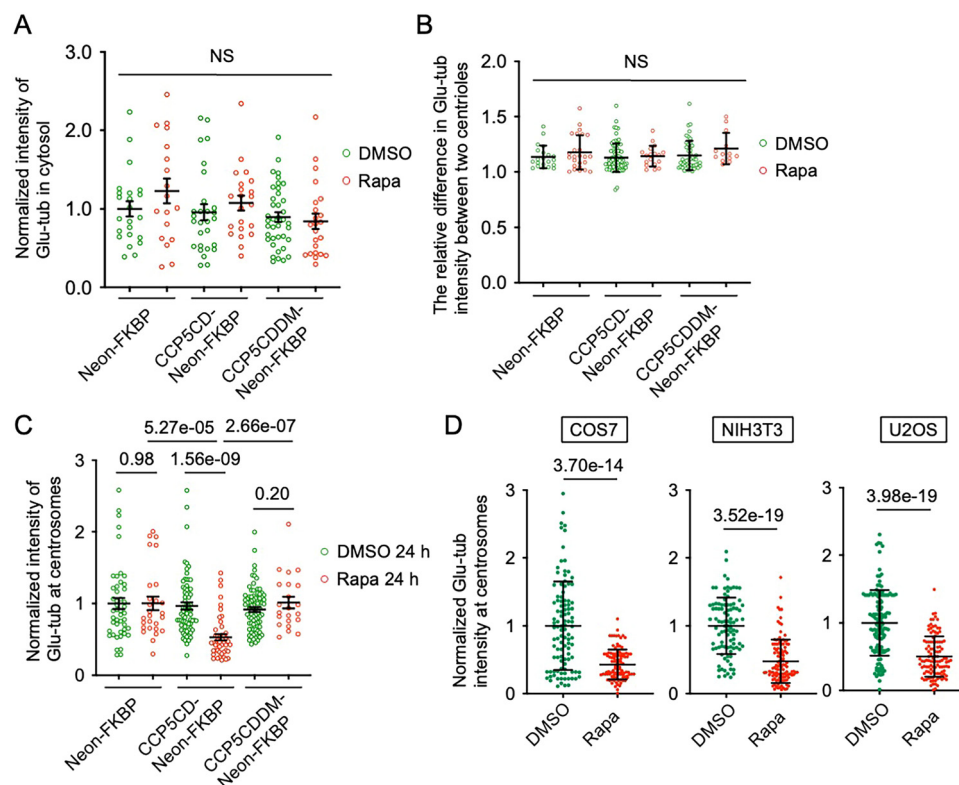


Figure EV1. Specific glutamylation reduction occurs at two centrioles in three different cell types.

(A–C) COS7 cells co-transfected with Ce3-FRB-CEP170C and the indicated constructs were treated with either 0.1% DMSO (green circles) or 100 nM rapamycin (Rapa, red circles) for 1 h in (A, B) or for 24 h in (C). Treated cells were immunostained with GT335 antibody to assess the level of glutamylated tubulin in cytosol (A); the relative difference in glutamylated tubulin intensity between two centrioles (B); and the normalized intensity of centrosomal glutamylation in (C). Data are presented as mean $\pm$ SEM. n (from left to right) = 23, 18, 28, 22, 39, and 23 cells in (A); 21, 25, 78, 18, 46, and 13 cells in (B); 47, 27, 71, 46, 85, and 22 cells in (C), collected from 3 independent experiments. Statistical significance was determined using a one-way ANOVA test. “NS” indicates no significant difference among groups in (A, B). Student’s t tests were performed, and P values are indicated in (C). (D) COS7, NIH3T3, and U2OS cells were transfected with Ce3-FRB-CEP170C and CCP5CD-Neon-FKBP. Transfected cells were treated with 0.1% DMSO (green) or rapamycin (100 nM) for 30 min. The normalized level of tubulin glutamylation at centrosomes under the indicated conditions is shown. Data (black) represent the mean $\pm$ SD. n = 105, 107, 97, 113, 133, and 112 cells from left to right. Three independent experiments. Student’s t tests were performed, and P values are indicated.

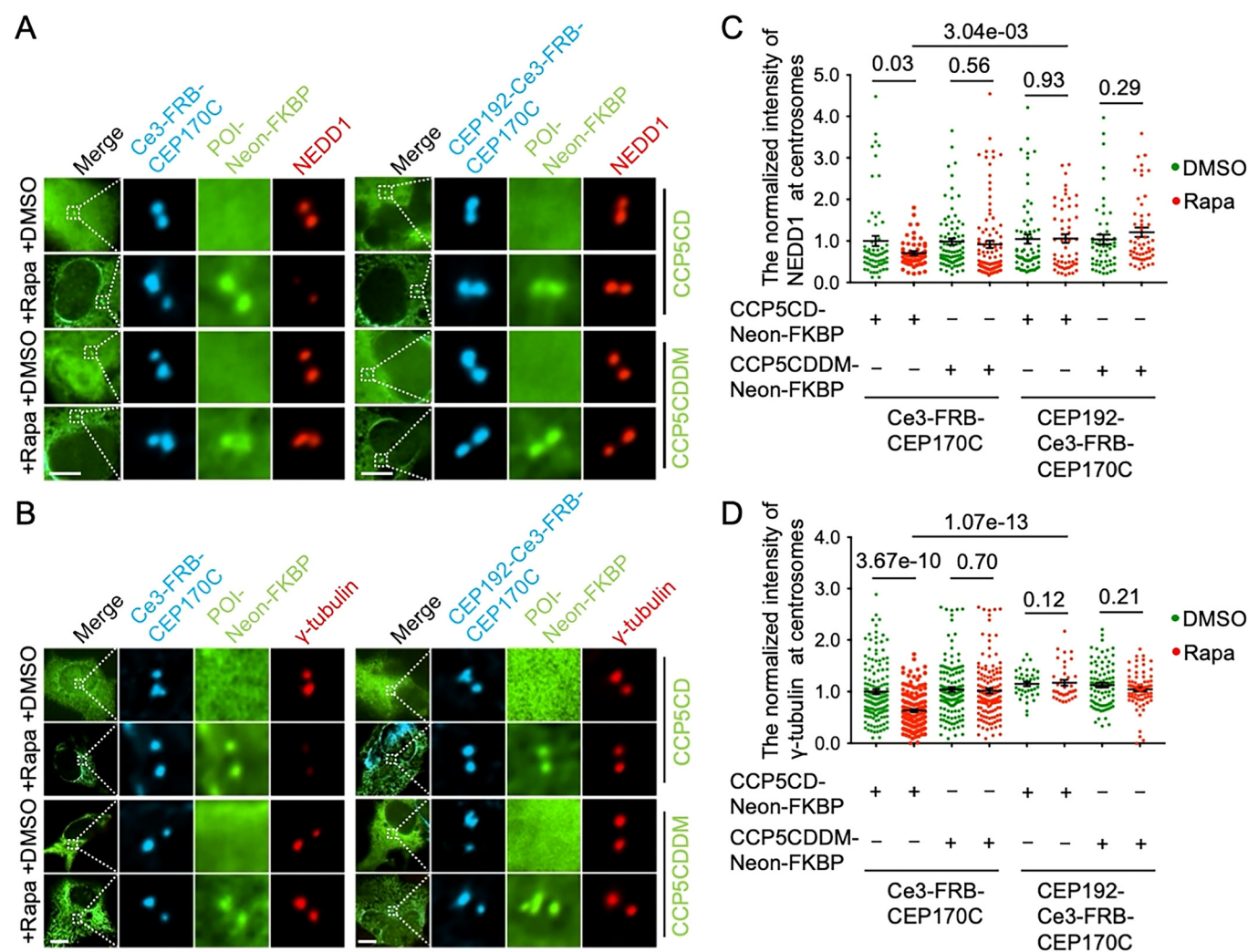


Figure EV2. CEP192 is sufficient to recruit NEDD1 and γ -tubulin to hypoglutamylylated centrosomes.

(A, B) COS7 cells co-transfected with the indicated constructs were treated with DMSO (0.1%) or Rapa (100 nM rapamycin) for 1 h. Following treatment, cells were immunostained with antibodies against NEDD1 (A) and γ -tubulin (B), respectively. Scale bar, 10 μ m. (C, D) Quantification of the normalized intensity of NEDD1 (C) in cells from (A) and γ -tubulin (D) in cells from (B). Data represent as mean $\pm$ SEM. n (from left to right) = 62, 51, 84, 101, 67, 55, 53, and 54 cells in (C); 143, 152, 134, 135, 37, 36, 85, and 69 cells in (D); 3–6 independent experiments. Students' t tests were performed, and P values are indicated.