

Expanded View Figures

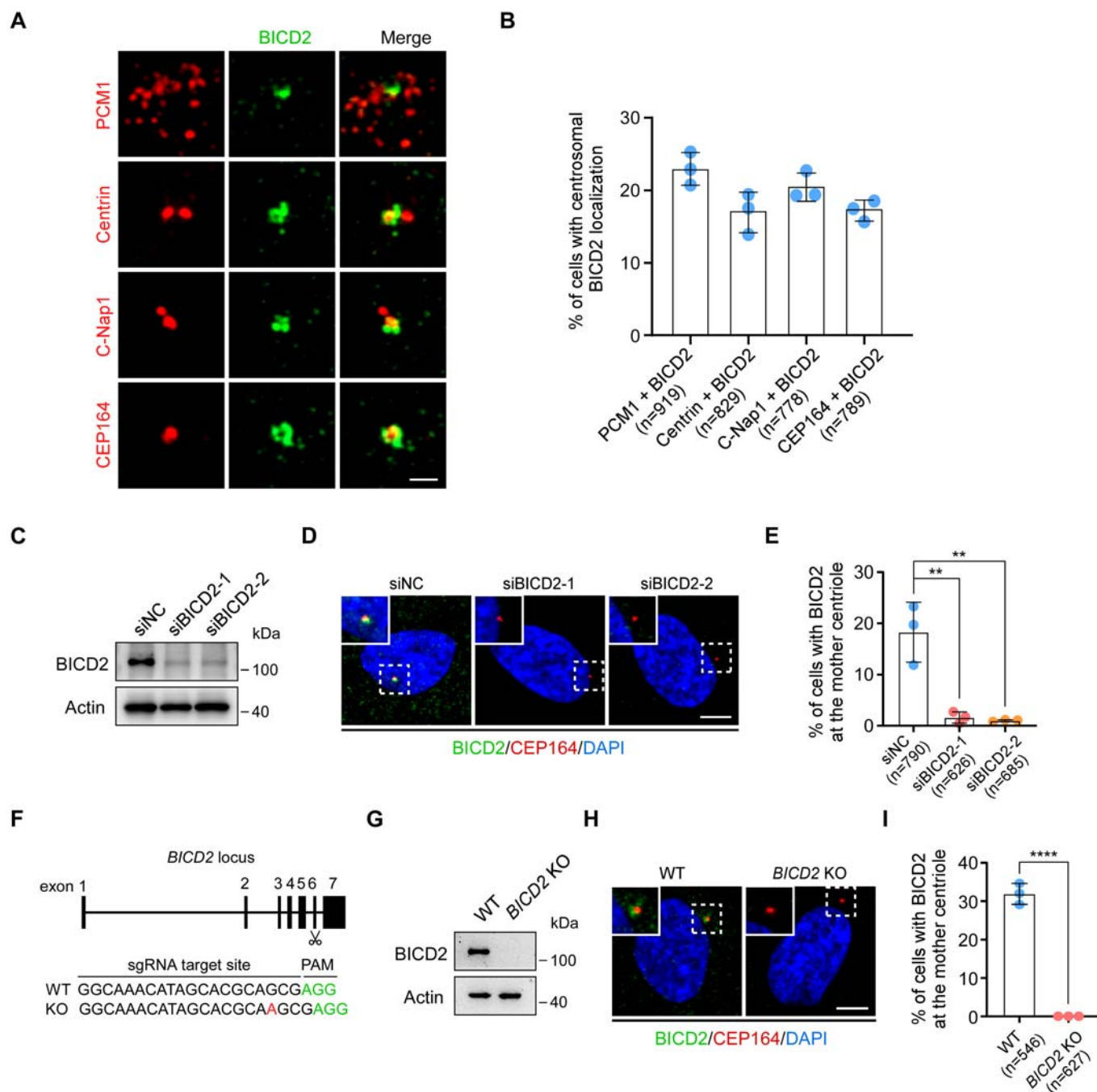


Figure EV1. Localization of BICD2 and verification of BICD2 antibody.

(A) Immunostaining images of RPE-1 cells stained with BICD2 antibody in conjunction with antibodies against PCM1, Centrin, C-Nap1, or CEP164. RPE-1 cells were cultured in normal serum medium. Scale bar, 1 μ m. (B) Quantification analysis of the percentage of cells showing BICD2 localization to PCM1, Centrin, C-Nap1, or CEP164. (C-E) RPE-1 cells transfected with non-targeting control (NC) or *BICD2* siRNAs for 48 h were subjected to Western blotting or immunofluorescence. Western blot analysis of BICD2 protein (C). Confocal images of RPE-1 cells stained with anti-BICD2 and anti-CEP164 antibodies (D). Scale bar, 5 μ m. Quantification analysis of the percentage of cells with BICD2 at the mother centriole (E). P (siNC vs. siBICD2-1) = 0.0083, P (siNC vs. siBICD2-2) = 0.0069. (F) Schematic representation of the CRISPR-Cas9 targeting site in the *BICD2* locus and the genotype of *BICD2* knockout RPE-1 cells. (G-I) Western blot analysis (G) and immunofluorescence analysis (H, I) of wild-type and *BICD2* knockout RPE-1 cells. Scale bar, 5 μ m. P (WT vs. *BICD2* KO) < 0.0001. Actin was served as a loading control. DNA was stained by DAPI. n, the number of total cells calculated. Data were presented as mean $\pm$ SD from three independent biological repeats. Student's t -test; ** P < 0.01, **** P < 0.0001.

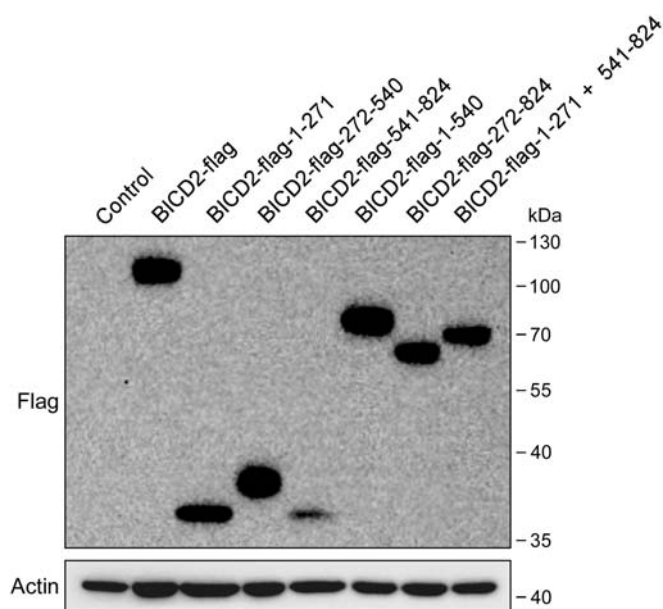


Figure EV2. The overexpression efficiency of full-length BICD2 and BICD2 truncation mutants.

Western blot analysis of the Flag protein in RPE-1 cells infected with lentiviruses carrying the indicated plasmids. Actin was used as a loading control.

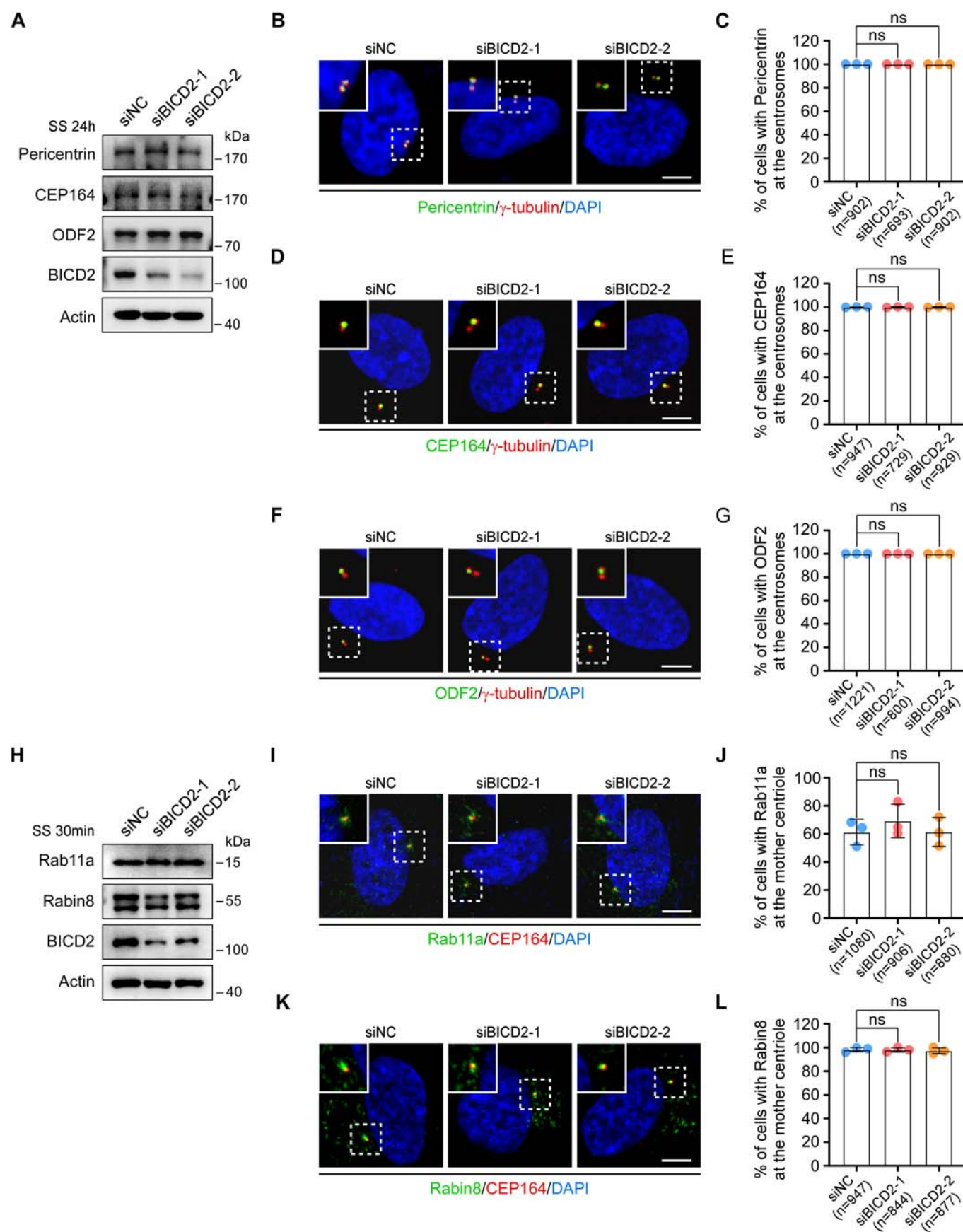


Figure EV3. BICD2 knockdown does not affect centrosome integrity, distal appendages assembly, or ciliary vesicle formation.

(A–G) RPE-1 cells transfected with control or *BICD2* siRNAs for 48 h were treated with serum starvation for an additional 24 h, and then subjected to Western blotting or immunofluorescence. (H–L) RPE-1 cells transfected with the indicated siRNAs for 48 h were treated with serum starvation for another 30 min, and then applied for Western blotting or immunofluorescence. Western blot analysis for the indicated proteins (A, H). Actin, a loading control. Confocal images of RPE-1 cells stained with antibodies against the indicated proteins (B, D, F, I, K). DNA was stained by DAPI. Scale bars, 5 μ m. Quantification analyses of the percentage of cells with Pericentrin, CEP164, or ODF2 at the centrosomes (C, E, G), or cells with Rab11a or Rabin8 at the mother centriole (J, L). *n*, the number of total cells calculated. Data were from three independent biological repeats and presented as mean $\pm$ SD. Student's *t*-test; ns not significant.

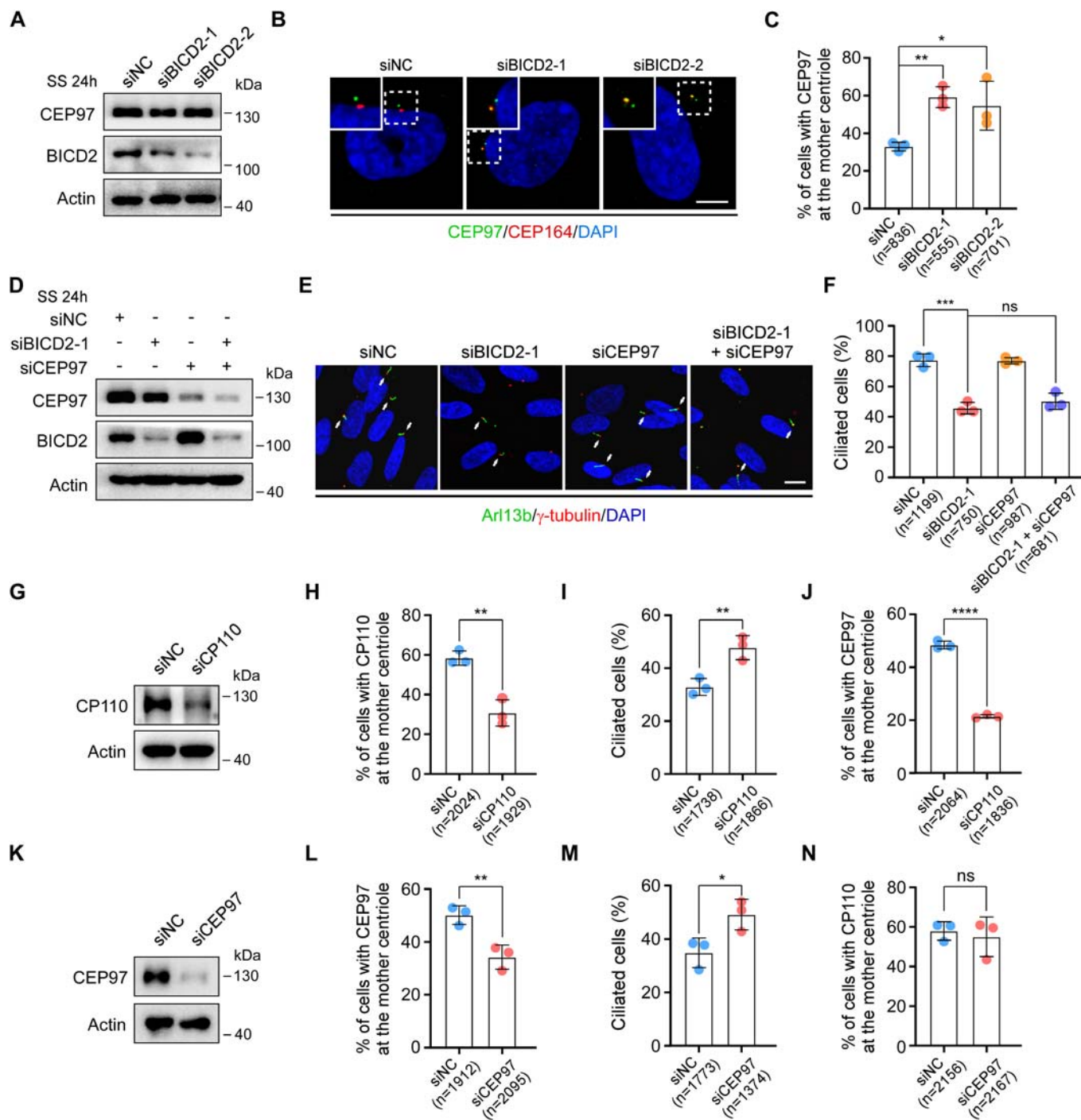


Figure EV4. CEP97 does not participate in the regulation of ciliogenesis mediated by BICD2.

(A–F) RPE-1 cells transfected with control or *BICD2* siRNAs for 48 h were treated with serum starvation for an additional 24 h, and then subjected to Western blotting or immunofluorescence. Western blot analysis of CEP97 and BICD2 proteins (A, D). Immunofluorescence images of RPE-1 cells stained with antibodies against CEP97 and CEP164 (B). Scale bar, 5 μ m. Quantification analysis of the percentage of cells with CEP97 at the mother centriole (C). P (siNC vs. siBICD2-1) = 0.0015, P (siNC vs. siBICD2-2) = 0.0465. Confocal images of RPE-1 cells stained with anti-Arl13b and anti- γ -tubulin antibodies (E). Cilia are indicated by white arrows. Scale bar, 10 μ m. Quantification analysis of the percentage of ciliated cells (F). P (siNC vs. siBICD2-1) = 0.0006, P (siBICD2-1 vs. siBICD2-1 + siCEP97) = 0.8818. (G–N) RPE-1 cells transfected with the indicated siRNAs for 48 h were applied for Western blotting or immunofluorescence. Immunoblotting of the indicated proteins (G, K). Quantification analyses of the percentage of cells with CP110 at the mother centriole (H, N), or ciliated cells (I, M), or cells with CEP97 at the mother centriole (J, L). CP110 at the mother centriole (H), P (siNC vs. siCP110) = 0.0031; ciliated cells (I, M), P (siNC vs. siCP110, siNC vs. siCEP97) = 0.0097, 0.036; CEP97 at the mother centriole (J, L), P (siNC vs. siCP110) < 0.0001, P ((L), siNC vs. siCEP97) = 0.0089. Actin was used as a loading control. DNA was stained by DAPI. n , the number of total cells calculated. Data were presented as mean $\pm$ SD from three independent biological repeats. Student's t -test; ns not significant; * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

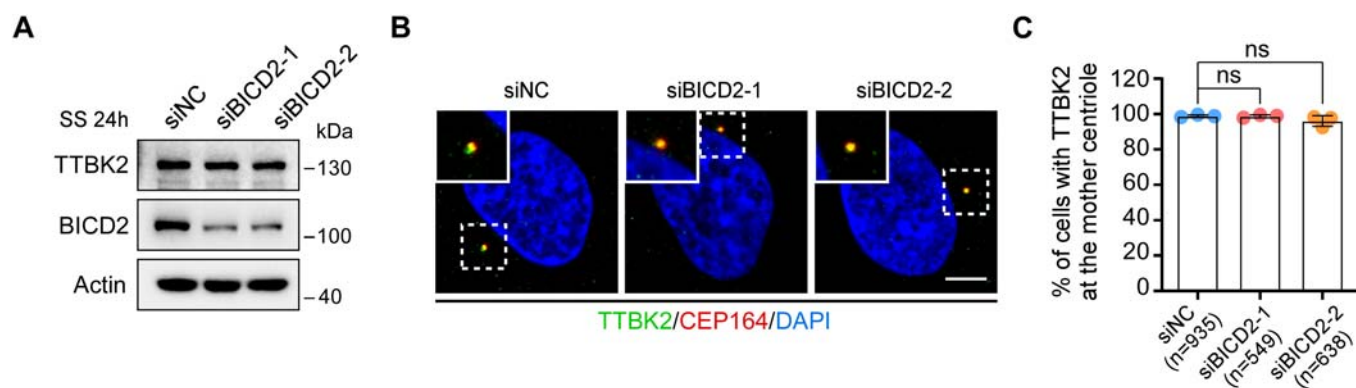
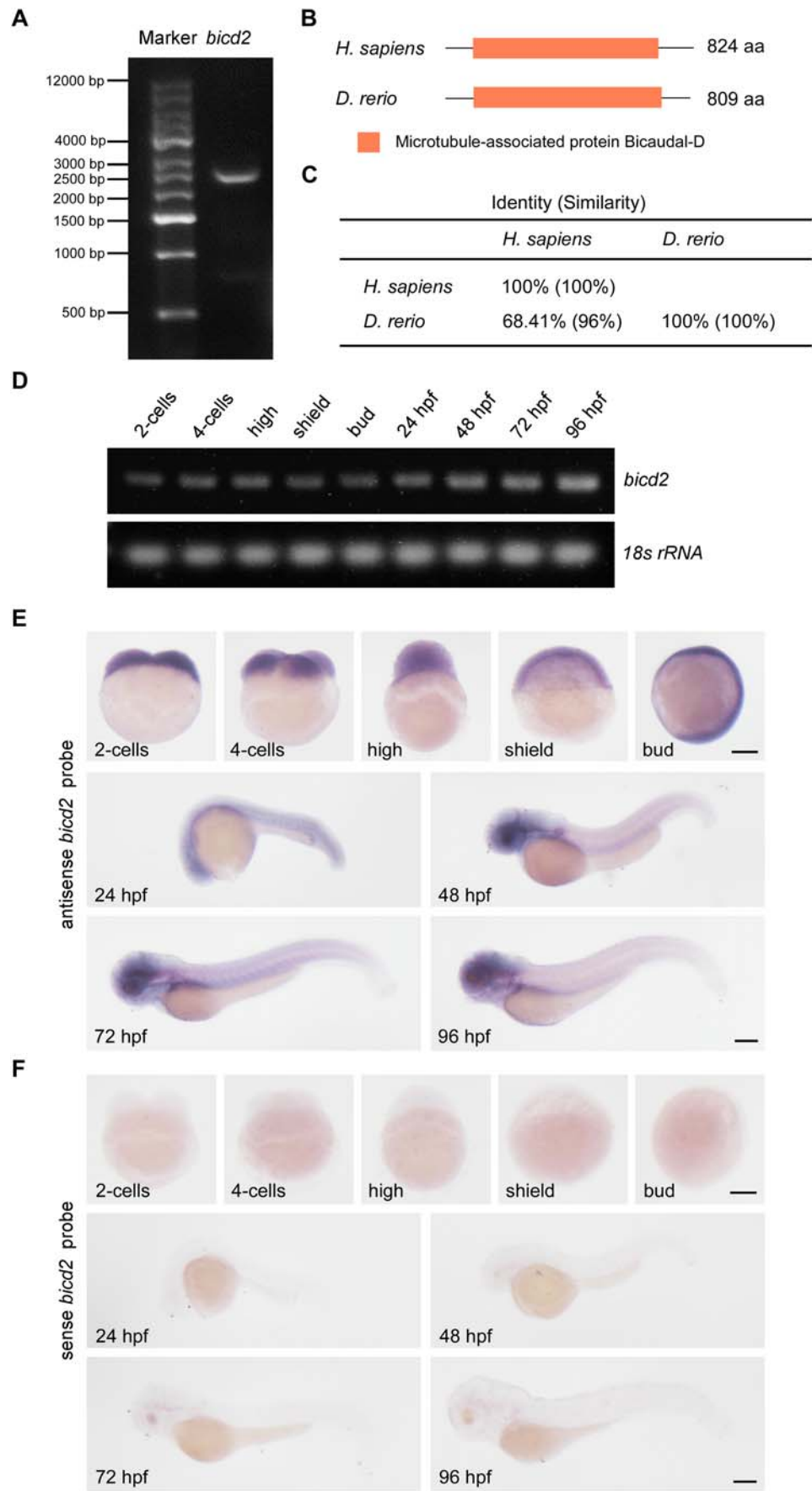


Figure EV5. Depletion of BICD2 does not affect the recruitment of TTBK2 to the mother centriole.

(A–C) RPE-1 cells transfected with control or *BICD2* siRNAs for 48 h were treated with serum starvation for an additional 24 h, and subsequently subjected to Western blotting or immunofluorescence. Western blot analysis of TTBK2 and BICD2 proteins (A). Actin, a loading control. Confocal images of RPE-1 cells stained with antibodies against TTBK2 and CEP164 (B). DNA was stained by DAPI. Scale bar, 5 μ m. *n* the number of total cells calculated. Quantification analysis of the percentage of cells with TTBK2 at the mother centriole (C). Data were from three independent biological repeats and shown as mean $\pm$ SD. Student's *t*-test; ns not significant.



**Figure EV6. Molecular characterization of zebrafish *bicd2* gene.**

(A) Cloning of zebrafish *bicd2* by reverse transcription PCR. (B, C) Schematic comparison of BICD2 amino acid sequences from the indicated species. The conserved microtubule-associated protein Bicaudal-D domains are shown in orange-filled bars. (D) Reverse transcription PCR analysis of *bicd2* mRNA at the different embryonic stages in zebrafish. 18s *rRNA* was used as a loading control. (E, F) Whole-mount in situ hybridization images of zebrafish embryos incubated with antisense (E) or sense (F) *bicd2* probes at the indicated developmental stages, shown in lateral view. hpf, hours post fertilization. Scale bars, 200 μ m. Source data are available online for this figure.