Supplemental material

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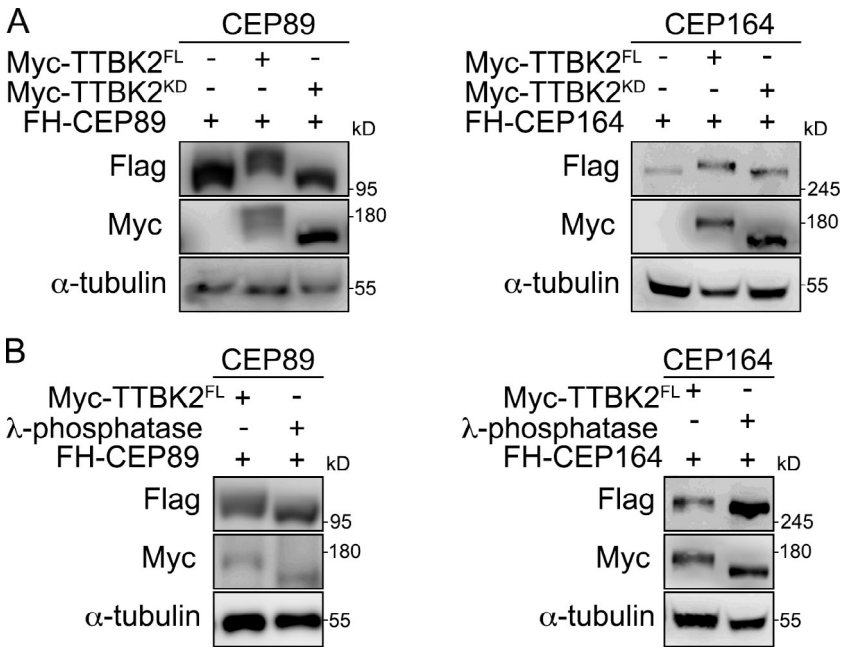

Figure S1. **TTBK2 coexpression causes phosphorylation-induced mobility shifts of CEP89 and CEP164. (A)** FH-CEP89 or FH-CEP164 was ectopically expressed in 293T cells along with Myc-TTBK2^{FL} or Myc-TTBK2^{KD}. Cell lysates were immunoprecipitated with anti-Flag antibody, and the phosphorylation-induced mobility shift was checked using the reducing gels. **(B)** Lysates of 293T cells transfected as indicated were treated with alkaline phosphatase (right) or DMSO (left). Western blots were performed to analysis phosphorylation-induced mobility shifts of CEP89 and CEP164 in the reducing gels.

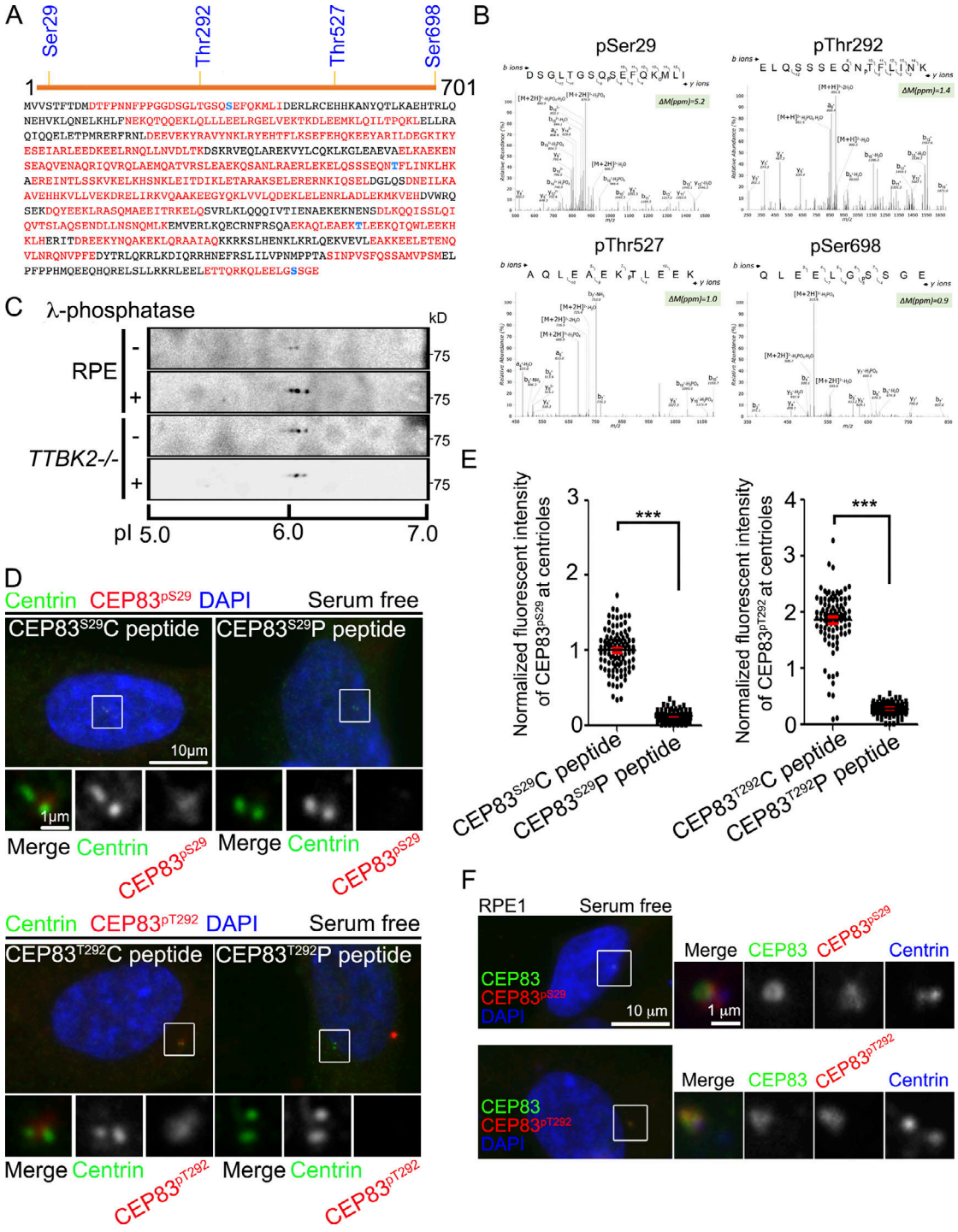

Figure S2. **CEP83 is phosphorylated by TTBK2 at Ser29, Thr292, Thr527, and Ser698. (A)** Myc-TTBK2 was coexpressed with FH-CEP83 in 293T cells. CEP83 was then purified from 293T cells using M2 beads. After electrophoresis, the phosphorylated CEP83 band was cut, followed by mass spectrometry analyses. A representative coverage map of peptides (red) and CEP83 phosphorylation sites (blue) is shown. Four phosphorylation sites (Ser29, Thr292, Thr527, and Ser698) were found on CEP83 when TTBK2 was coexpressed. **(B)** MS/MS of quantified phosphopeptides spanning aa 21–36 (DSGLTGSQSEFQKMLI), 283–297 (ELQSSSEQNTFLINK), 520–513 (AQLEAEKTLEEK), and 692–701 (QLEELGSSGE) revealed four novel TTBK2-dependent CEP83 phosphorylation sites on Ser29, Thr292, Thr527, and Ser698. The results obtained by MS/MS were in biological triplicates. **(C)** Cells were serum starved for 2 d. Cell lysates were extracted from WT and *TTBK2*$^{-/-}$ RPE1 cells and analyzed by isoelectric focusing in the first dimension followed by SDS-PAGE in the second dimension. The gels were then transferred to a PVDF membrane for immunoblot analysis using anti-CEP83 antibodies. **(D)** Cells were serum starved for 2 d. Immunostaining was performed with two phospho-CEP83 antibodies (anti-phospho-CEP83^{Ser29} and anti-phospho-CEP83^{Thr292}) that were preincubated with the indicated peptides. Centrioles are stained by centrin antibody. DNA was stained using DAPI (blue). **(E)** The phosphorylated CEP83^{Ser29} and CEP83^{Thr292} signals at the centrioles were quantified. 200 cells were analyzed for each independent experiment. Error bars represent mean ± SEM; n = 3. ***, $P < 0.001$ (Student's t test). **(F)** RPE1 cells were serum starved for 2 d and stained with antibodies against CEP83 (green), phospho-CEP83^{Ser29} (CEP83^{pS29}; red), and phospho-CEP83^{Thr292} (CEP83^{pT292}; red). DNA was stained by DAPI (blue). Scale bar as indicated.

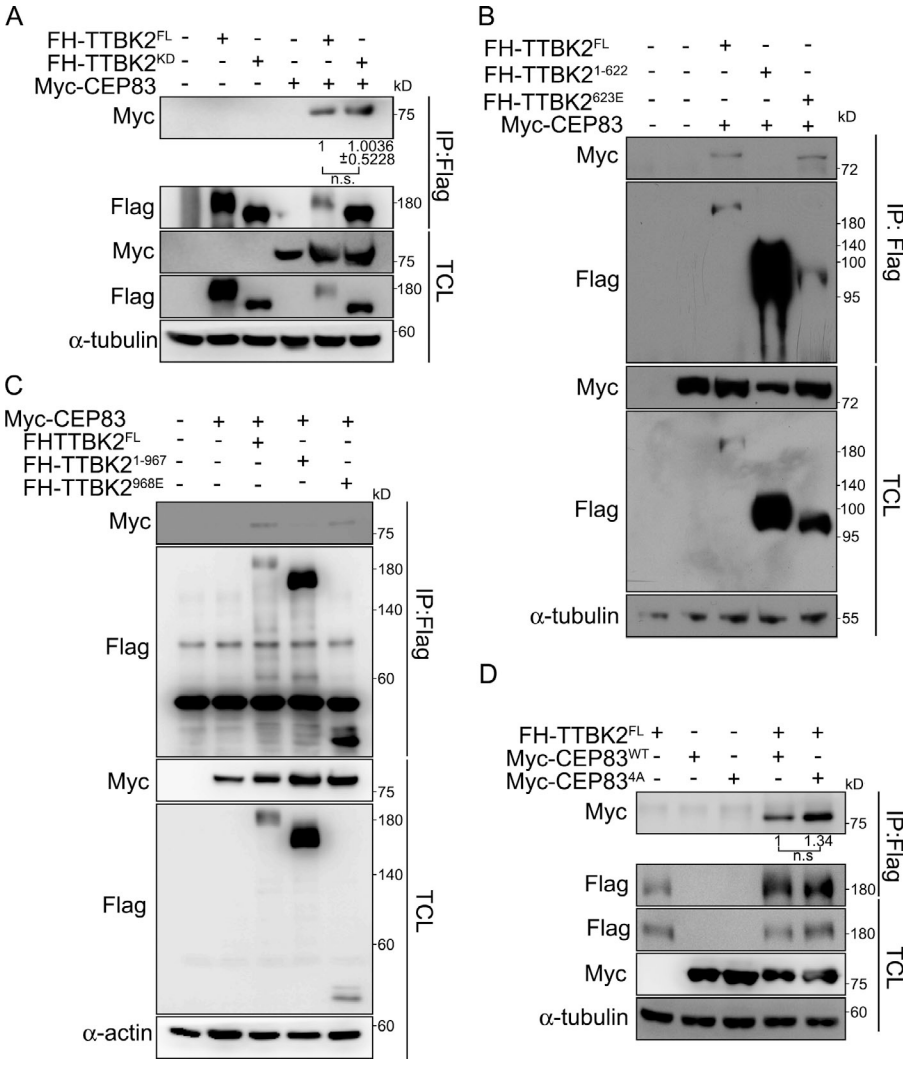

Figure S3. **TTBK2 interacts with CEP83 via its C-terminal region. (A–D)** 293T cells transfected with various expression constructs were analyzed by immunoprecipitation (IP) followed by Western blots with indicated antibodies. Relative amounts of CEP83 pulled down by TTBK2 were quantified and normalized to purified FH-TTBK2 n.s., not significant (Student's *t* test).

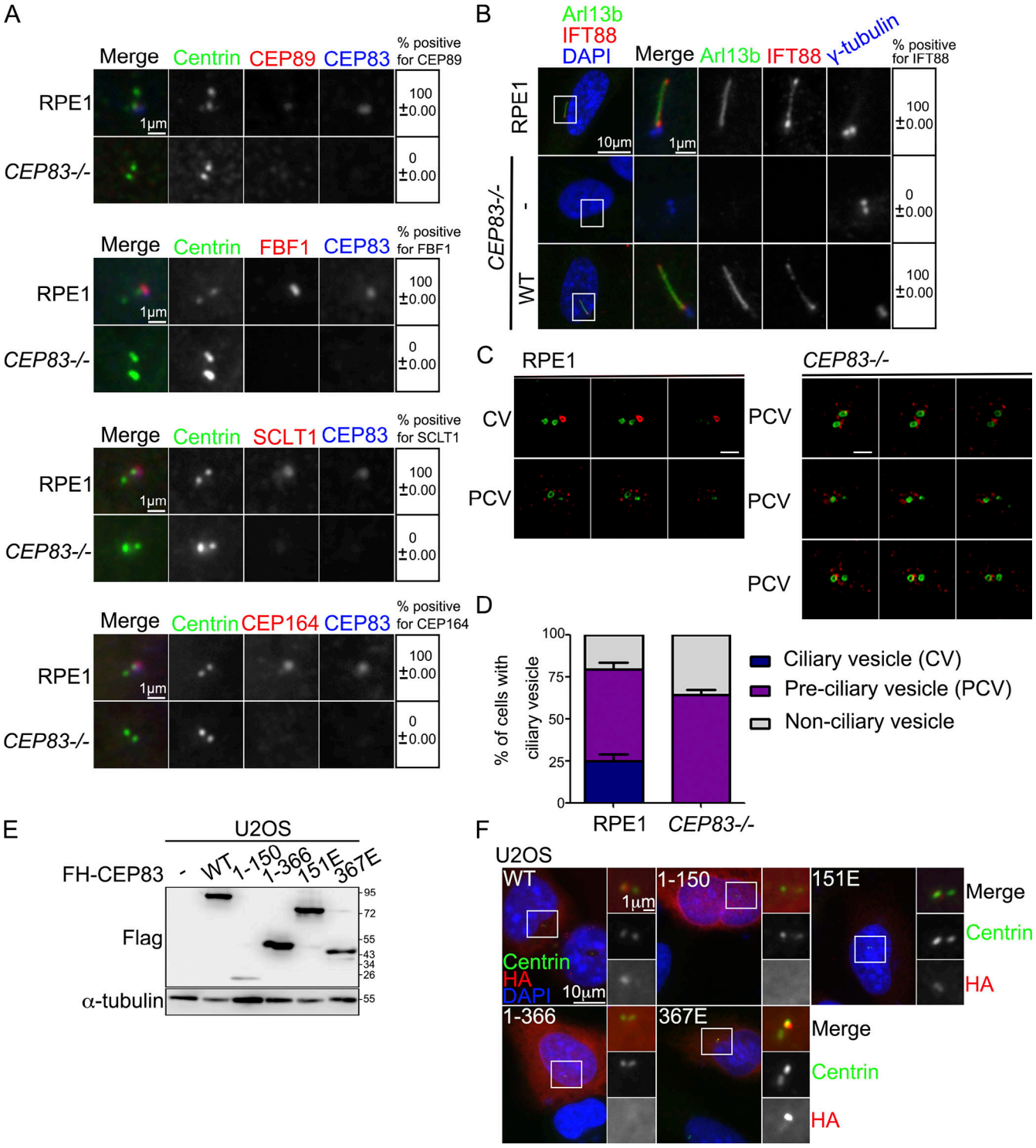

Figure S4. **CEP83 inactivation blocks DA assembly, the recruitment of IFT88 to the mother centrioles, and ciliary vesicle docking during ciliogenesis. (A and B)** WT and *CEP83*$^{-/-}$ cells were serum starved for 2 d and stained with antibodies as indicated. DNA was stained by DAPI (blue). Cells were also examined for the presence of indicated DAPs and IFT88. 200 cells were analyzed for each independent experiment. Error bars represent mean ± SEM; *n* = 3. **(C)** WT and *CEP83*$^{-/-}$ cells were serum-starved for 6 h. Cells were stained with antibodies against pGlu-Tu and myosin-Va. Images were acquired by 3D-SIM. Scale bar, 1 µm. **(D)** The percentages of cells with different types of myosin-Va staining patterns were quantified. More than 150 cells were analyzed for each independent experiment. Error bars represent mean ± SEM; *n* = 3. **(E and F)** Various FH-CEP83 mutants were transiently expressed in U2OS cells. Immunostaining and immunoblots were performed using antibodies as indicated.

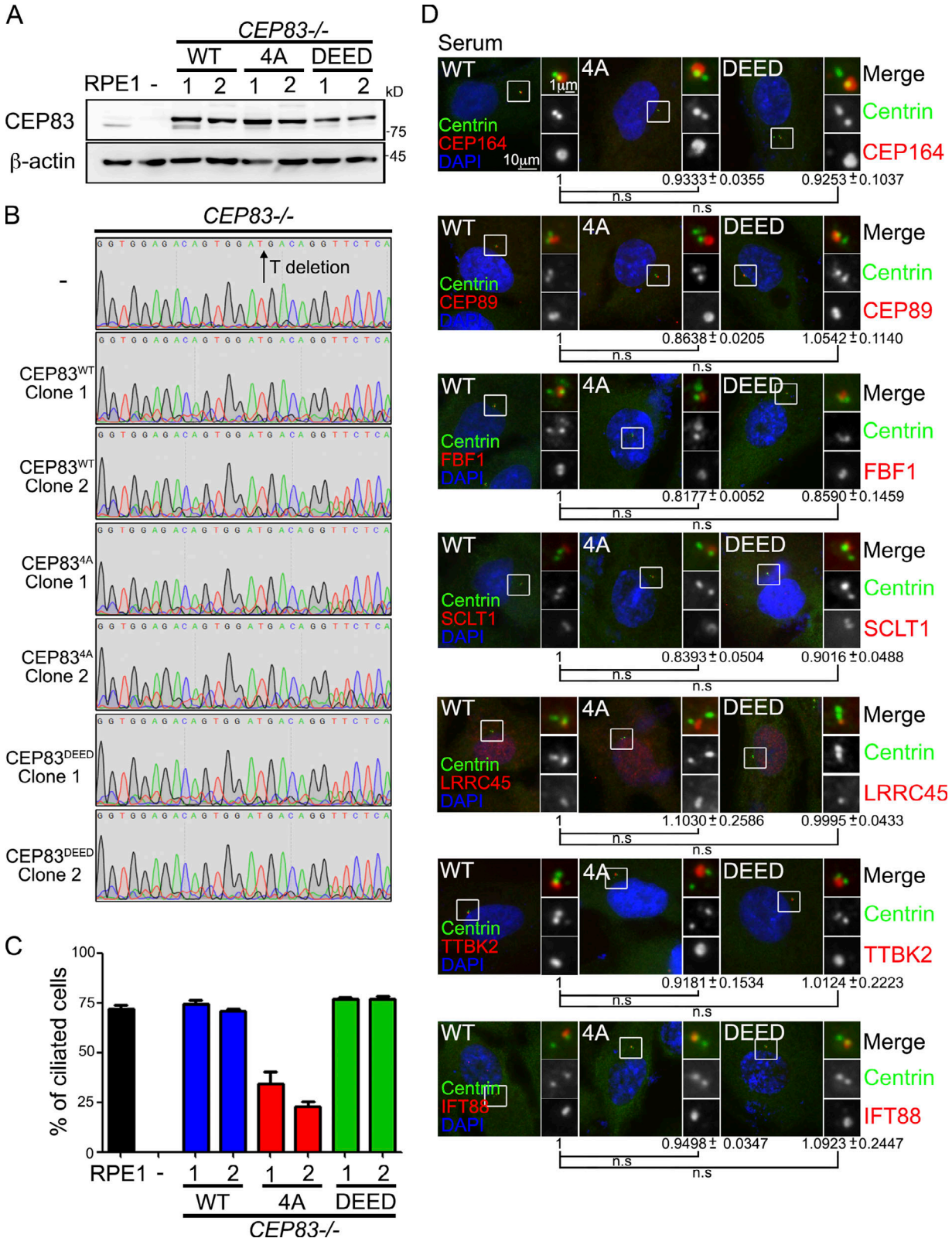

Figure S5. **CEP83 phosphorylation promotes cilia formation without affecting DA assembly. (A)** Various FH-CEP83 mutants were stably expressed in *CEP83*$^{-/-}$ cells. Various CEP83-expressing cell lines were established through clonal propagation from a single cell. Western blots were performed to confirm the expression of CEP83. **(B)** Sequences analysis of *CEP83* alleles in CEP83-expressing cell lines. All clones contained a base pair (T) deletion on both alleles, indicating the *CEP83* alleles were edited. **(C)** Cells were serum starved for 2 d. The percentage of ciliated cells was measured and quantified by Arl13b staining. **(D)** Proliferating cells were stained with antibodies as indicated. DNA was stained with DAPI (blue). The intensity of CEP164, CEP89, FBF1, SCLT1, LRRC45, TTBK2, and IFT88 at the centrioles was also quantified. In C and D, >150 cells were analyzed for each independent experiment. Error bars represent mean ± SEM; *n* = 3. n.s., not significant.