

Supplementary Information

CFAP100 forms phase-separated condensates required for ciliary transition zone assembly

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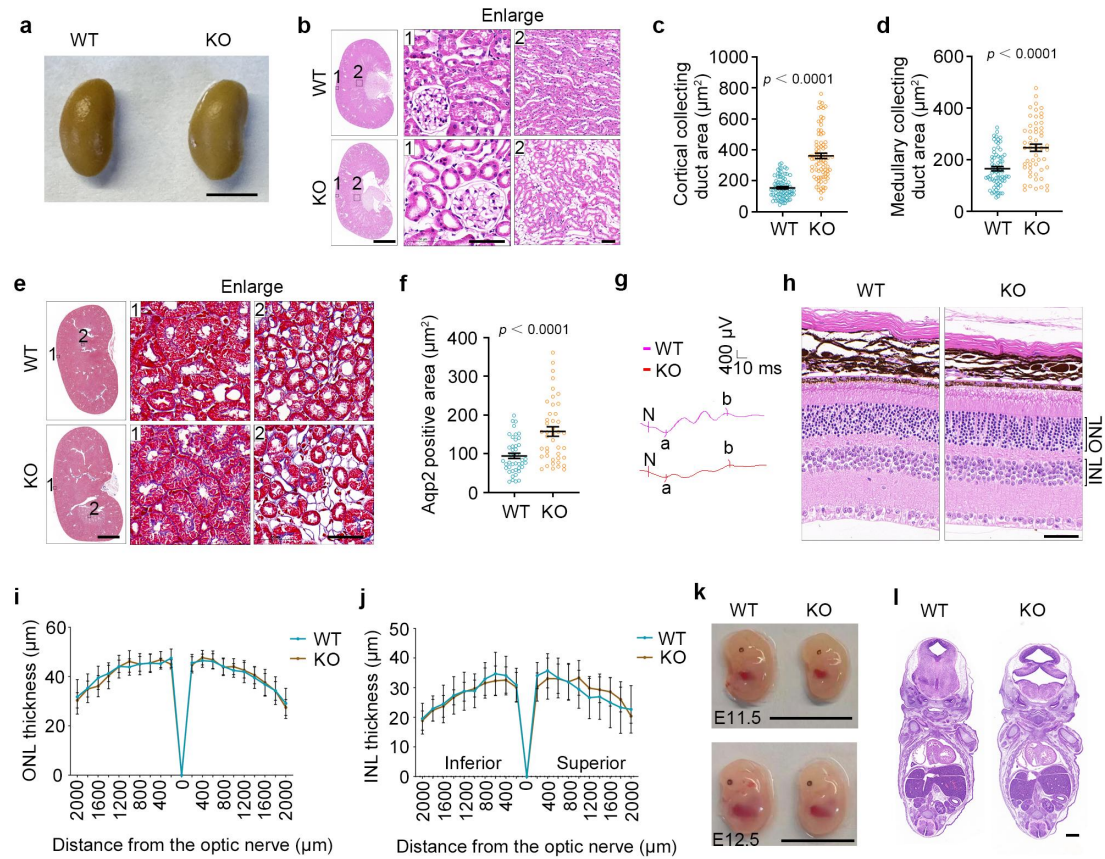
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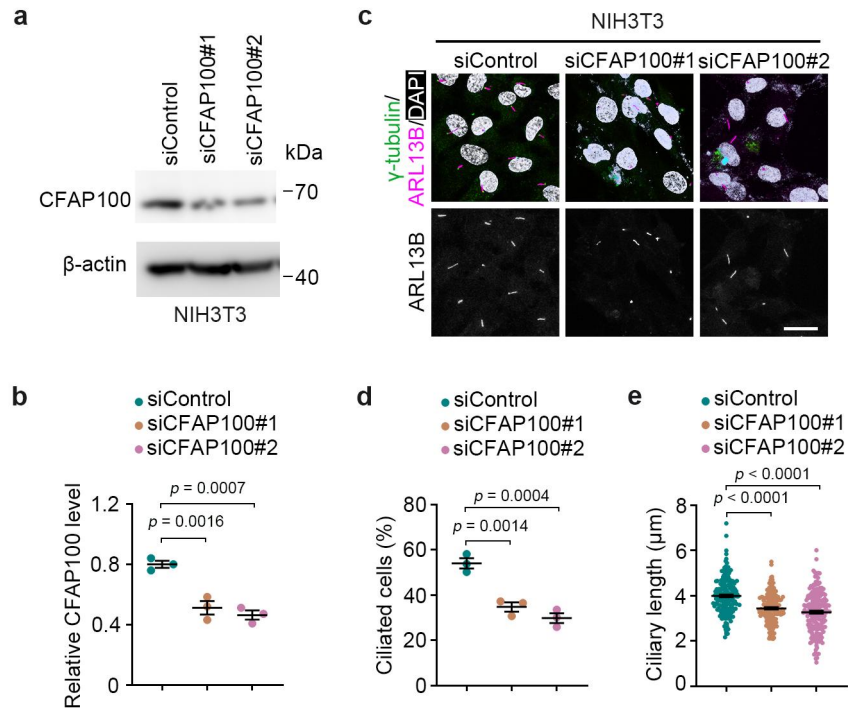
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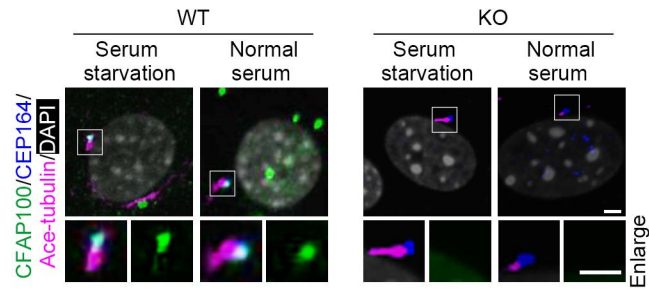


Supplementary Figure 1 ***Cfap100* knockout mice display defects in multiple organs.**

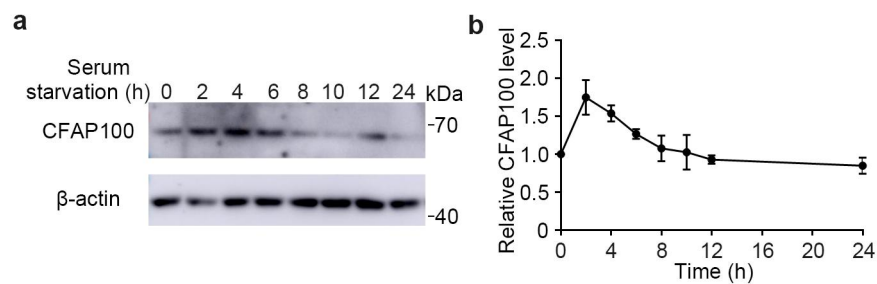
a Gross appearance of kidneys from 12-month-old wild-type (WT) and *Cfap100* knockout (KO) mice. Scale bar, 5 mm. **b-d** HE staining of kidneys from 12-month-old WT and KO mice (**b**). Scale bars: 2 mm (overview) and 50 μ m (enlarge). Quantification of the tubular luminal area in the cortex (**c**, $n = 80$ ducts from 3 mice) and medulla (**d**, $n = 80$ ducts from 3 mice). **e** Masson's trichrome staining of kidneys from 12-month-old WT and KO mice. Scale bars: 2 mm (overview) and 50 μ m (enlarge). **f** Quantification of AQP2-positive principal collecting duct area. $n = 50$ ducts from 3 mice per group. **g** ERG analysis of 12-month-old WT and KO mice. **h-j** HE staining of eyes from 12-month-old WT and KO mice (**h**). Scale bar, 50 μ m. Quantification of the thickness of outer nuclear layer (ONL, **i**) and inner nuclear layer (**j**). **k** Whole-mount images of E11.5 and E12.5 mouse embryos. **l** HE staining of sections from E12.5 mouse embryos. Data are presented as mean $\pm$ SEM. P values were determined using an unpaired two-tailed Student's t-test (**c**, **d**, **f**). Source data are provided as a Source Data file.



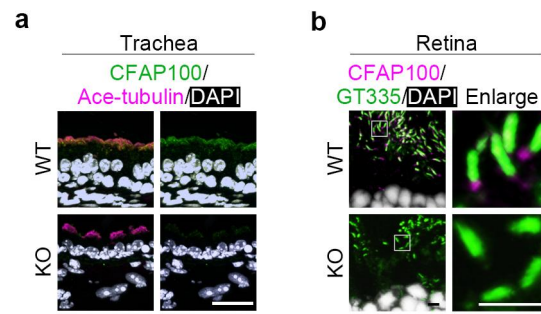
Supplementary Figure 2 CFAP100 promotes the initiation of ciliogenesis. a-b Immunoblot analysis of CFAP100 expression (**a**) and quantification of its relative level (**b**, $n = 3$ independent experiments) in NIH3T3 cells transfected with control or CFAP100-specific siRNAs in serum-free medium for 48 h. **c-e** Immunofluorescence microscopy (**c**) and quantification of percentage of ciliated cells (**d**, $n = 3$ independent experiments) and ciliary length (**e**, $n = 200$ cilia from 3 independent experiments) for MEFs cultured in serum-free medium for 48 h and stained with antibodies against ARL13B and γ -tubulin and DAPI. Scale bar, 10 μ m. Data are presented as mean $\pm$ SEM. P values were determined one-way ANOVA with Dunnett's post-hoc analysis (**b**, **d**, and **e**). Source data are provided as a Source Data file.



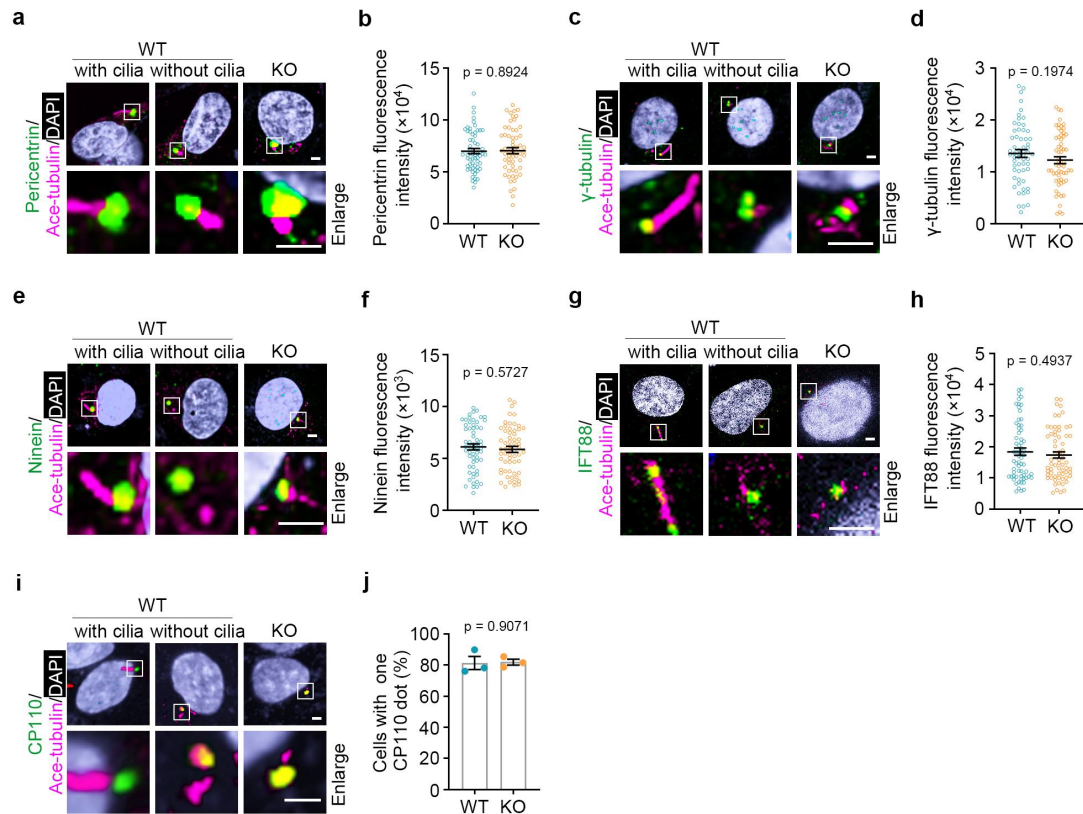
Supplementary Figure 3 **Validation of the anti-CFAP100 antibody specificity using MEFs.** WT and *Cfap100* KO MEFs were serum-starved for 12 h to induce ciliogenesis. Immunofluorescence staining with the home-made anti-CFAP100 antibody shows specific signal in WT MEFs, while no signal is detected in *Cfap100* KO MEFs under normal serum or serum-starvation conditions. Scale bars, 3 μ m. The experiment was independently repeated three times with consistent results.



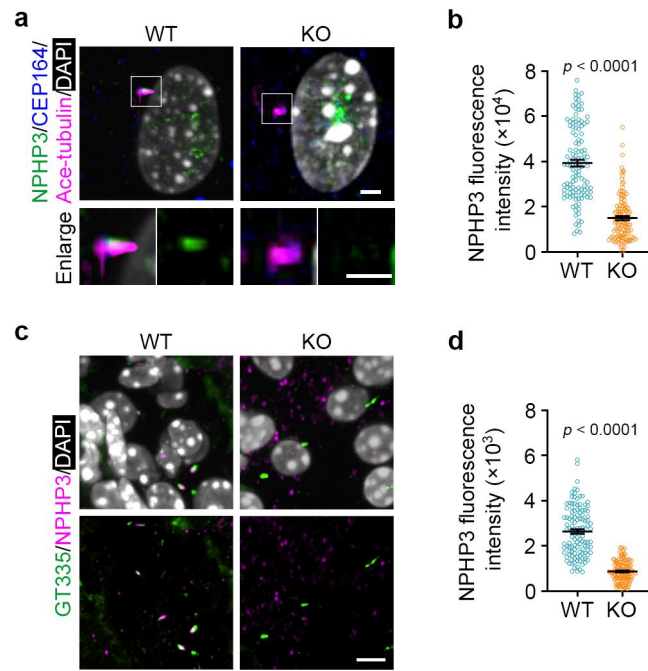
Supplementary Figure 4 **The level of CFAP100 changes during ciliogenesis. a-b** Immunoblot analysis of CFAP100 expression (**a**) and quantification of its relative levels (**b**, $n = 3$ independent experiments) in RPE1 cells following serum withdrawal. Source data are provided as a Source Data file.



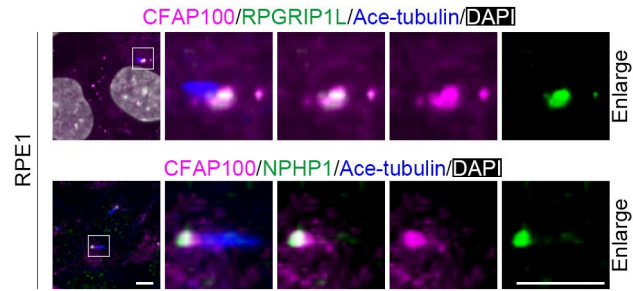
Supplementary Figure 5 **Subcellular localization of CFAP100 in the trachea and retina.** **a** Immunofluorescence images showing CFAP100 localization in the tracheal epithelial cilia of WT and *Cfap100* KO mice. Scale bar, 20 μ m. **b** Immunofluorescence analysis of CFAP100 in the retinal photoreceptor cilia of WT and *Cfap100* KO mice. Scale bar, 5 μ m. The experiment was independently repeated three times with consistent results.



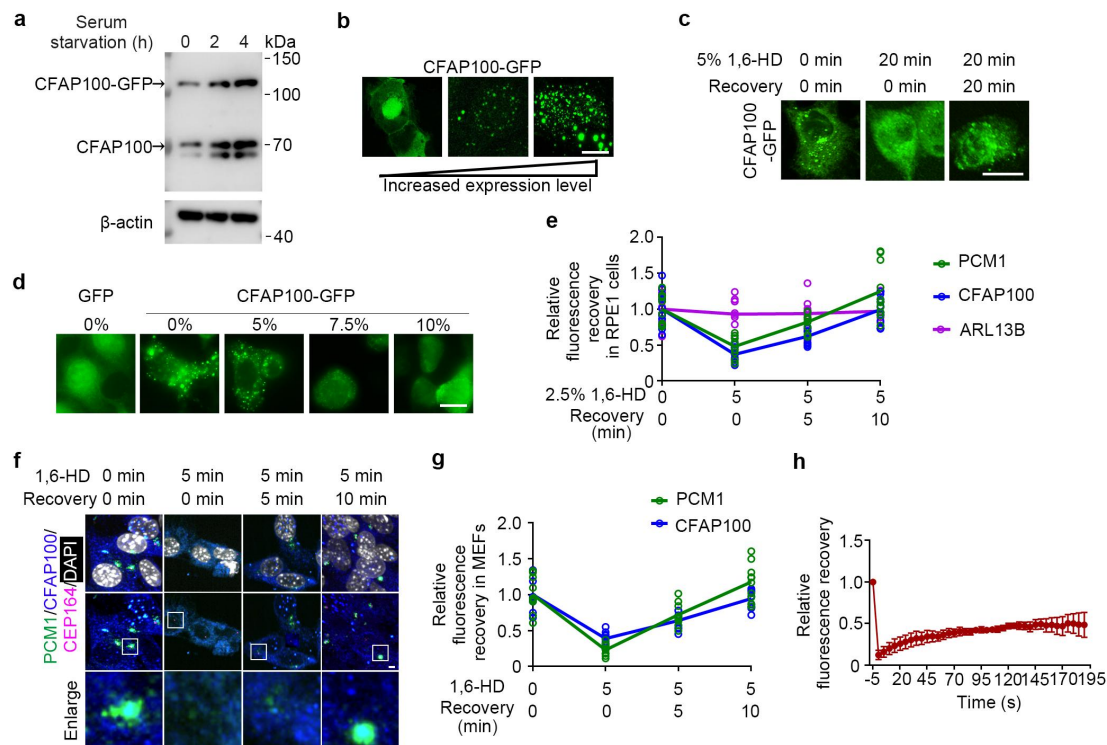
Supplementary Figure 6 CFAP100 functions in the recruitment of ciliogenesis related proteins. **a-b** Immunofluorescence microscopy (**a**) and quantification of the fluorescence intensity of pericentrin (**b**, $n = 60$ cells from 3 independent experiments) in WT and *CFAP100* KO RPE1 cells. Scale bar, 3 μ m. **c-d** Immunofluorescence microscopy (**c**) and quantification of the fluorescence intensity of γ -tubulin (**d**, $n = 60$ cells from 3 independent experiments) in WT and *CFAP100* KO RPE1 cells. Scale bar, 3 μ m. **e-f**. Immunofluorescence microscopy (**e**) and quantification of the fluorescence intensity of ninein (**f**, $n = 60$ cells from 3 independent experiments) in WT and *CFAP100* KO RPE1 cells. Scale bar, 3 μ m. **g-h**. Immunofluorescence microscopy (**g**) and quantification of the fluorescence intensity of IFT88 (**h**, $n = 60$ cells from 3 independent experiments) in WT and *CFAP100* KO RPE1 cells. **i-j**. Immunofluorescence microscopy (**i**) and quantification of the percentage of cells with one CP110 dot (**j**, $n = 3$ independent experiments) in WT and *CFAP100* KO RPE1 cells. WT and *CFAP100* KO RPE1 cells in a-j are all cultured in serum-free medium for 4 h and stained with indicated antibodies and DAPI. Data are presented as mean $\pm$ SEM. P values were determined using an unpaired two-tailed Student's t-test (**b**, **d**, **f**, **h**, **j**). Source data are provided as a Source Data file.



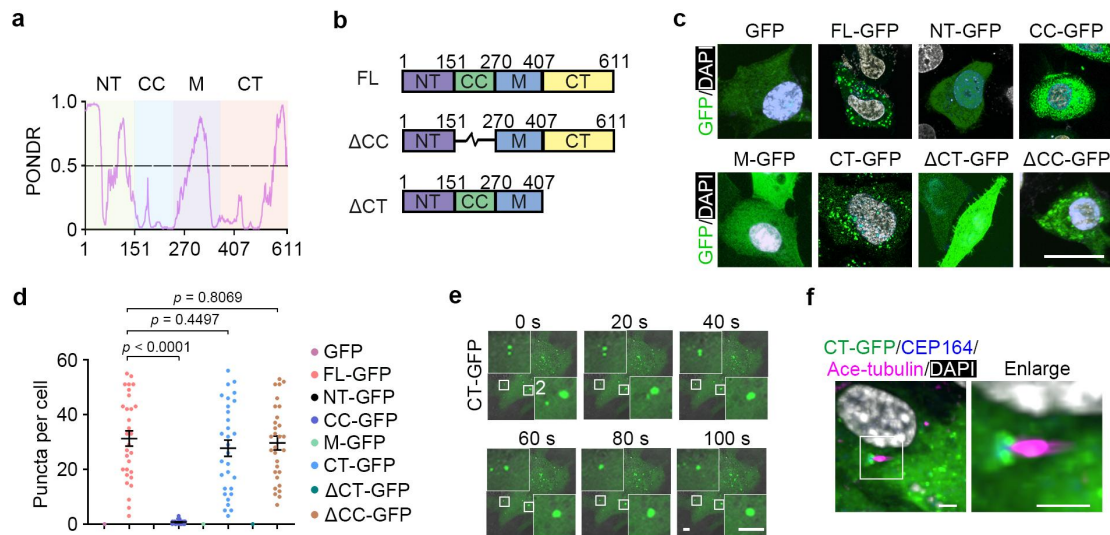
Supplementary Figure 7 **CFAP100 functions in the recruitment of NPHP3.** **a-b** Immunofluorescence microscopy (**a**) and quantification of the fluorescence intensity of NPHP3 (**b**, $n = 120$ cells from 3 independent experiments) in WT and *Cfap100* KO MEFs. Scale bar, 3 μm . **c-d** Immunofluorescence microscopy (**c**) and quantification of the fluorescence intensity of NPHP3 (**d**, $n = 120$ cells from 3 independent experiments) in the renal cilia of WT and *Cfap100* KO mice. Scale bar, 5 μm . Data are presented as mean $\pm$ SEM. P values were determined using an unpaired two-tailed Student's t-test (**b**, **d**). Source data are provided as a Source Data file.



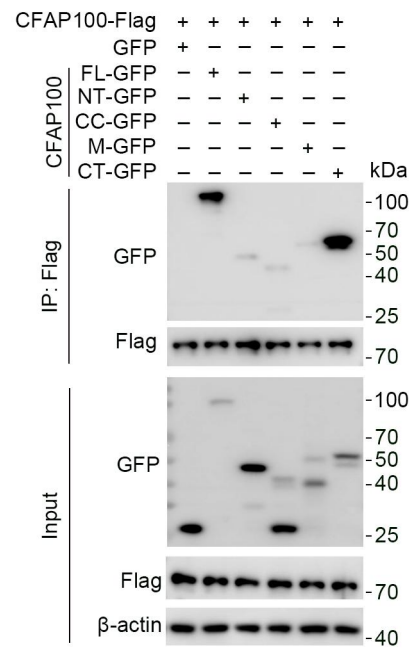
Supplementary Figure 8 **Subcellular localization of CFAP100 during ciliogenesis.** Immunofluorescence microscopy showing the co-localization of endogenous CFAP100 with RPGRIP1L and NPHP1 in RPE1 cells cultured in serum-free medium. Ace-tubulin indicates the ciliary axoneme. Scale bar, 5 μ m.



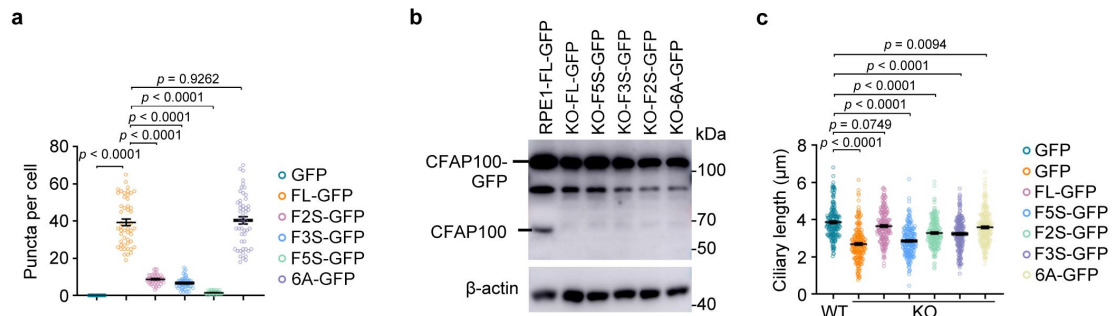
Supplementary Figure 9 Analysis of the phase separation property of CFAP100. **a** Immunoblot analysis of IMCD3 cells stably expressing CFAP100-GFP after serum starvation for the indicated time. The level of CFAP100-GFP expression is comparable to that of endogenous CFAP100. **b** Fluorescence microscopy of CFAP100-GFP droplets in serum-starved RPE1 cells. Scale bar, 20 μ m. **c** Fluorescence microscopy showing the dissolution and reformation of CFAP100-GFP droplets in HeLa cells following treatment with 5% 1,6-hexanediol for 20 min and subsequent washout. Scale bar, 20 μ m. **d** Dissolution of CFAP100-GFP condensates in HeLa cells treated with increasing concentrations of 1,6-hexanediol for 10 min. Scale bar, 20 μ m. **e** Quantification of the fluorescence intensity of PCM1, CFAP100, and ARL13B at the ciliary base upon 1,6-hexanediol treatment and recovery in RPE1 cells. $n = 10$ fields from 3 independent experiments. **f** Analysis of the phase separation property of endogenous CFAP100. MEFs were serum-starved for 4 h to induce ciliogenesis and then treated with 2.5% 1,6-hexanediol for 5 min. Cells were subsequently washed and incubated in serum-free medium for the indicated time (recovery time). PCM1 was used here as a positive control. Scale bar, 3 μ m. **g** Quantification of the fluorescence intensity of PCM1 and CFAP100 at the ciliary base upon 1,6-hexanediol treatment and recovery in MEFs. $n = 10$ fields from 3 independent experiments. **h** FRAP curve of CFAP100-GFP droplets in the ciliary base in RPE1 cells. Images were acquired every 5 s. The recovery curve represents data from 3 independent experiments.



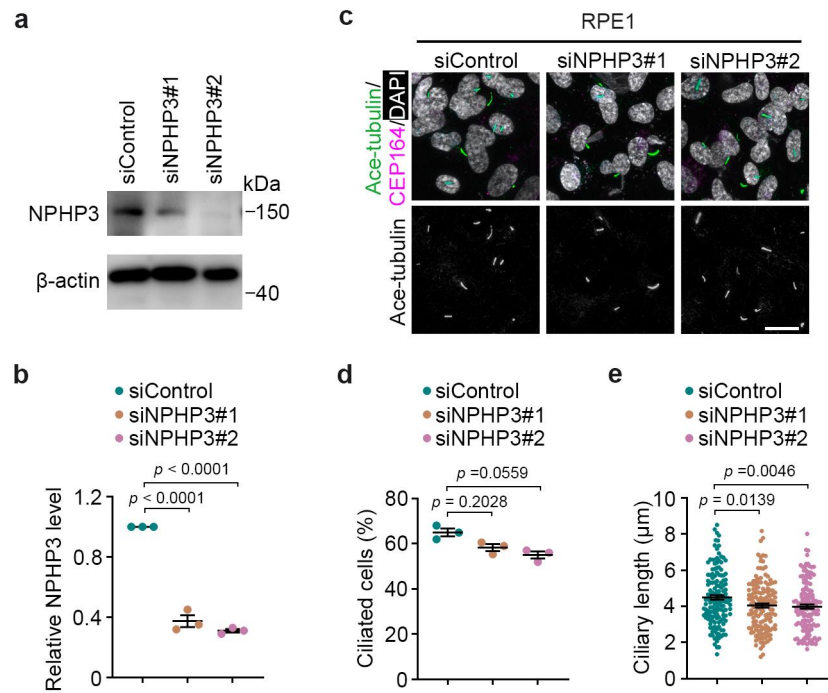
Supplementary Figure 10 Analysis of the phase separation property of the truncated mutants of CFAP100. **a** Prediction of IDRs in CFAP100 with PONDR (threshold $y = 0.5$; >0.5 : disordered, <0.5 : ordered). NT, N-terminus. CC, coiled coil. M, middle region. CT, C-terminus. **b** Schematic representation of CFAP100 and its truncated mutants. FL, full-length. **c-d** Fluorescence microscopy (**c**) and quantification (**d**) of droplet formation in RPE1 cells transfected with GFP, CFAP100-GFP, and the indicated mutants. Scale bar, 20 μm . **e** Time-lapse fluorescence microscopy of CFAP100 CT-GFP condensates undergoing rapid fusion in RPE1 cells. Insets show magnified fusion events. **f** Immunofluorescence staining of CFAP100 CT-GFP in RPE1 cells serum-starved for 4 h. CEP164 was used as a centrosomal marker. Scale bar, 3 μm . Data are presented as mean $\pm$ SEM. P values were determined one-way ANOVA with Dunnett's post-hoc analysis (**d**). Source data are provided as a Source Data file.



Supplementary Figure 11 **Interaction between full-length CFAP100 and truncation mutants in HEK293T cells.** Immunoprecipitation and immunoblotting analysis showing interactions between full-length CFAP100-Flag and various CFAP100-GFP truncation mutants in HEK293T cells. Lysates from HEK293T cells co-expressing CFAP100-Flag and the indicated CFAP100-GFP truncation mutants were subjected to immunoprecipitation with anti-Flag antibody, followed by immunoblotting with anti-GFP and anti-Flag antibodies.

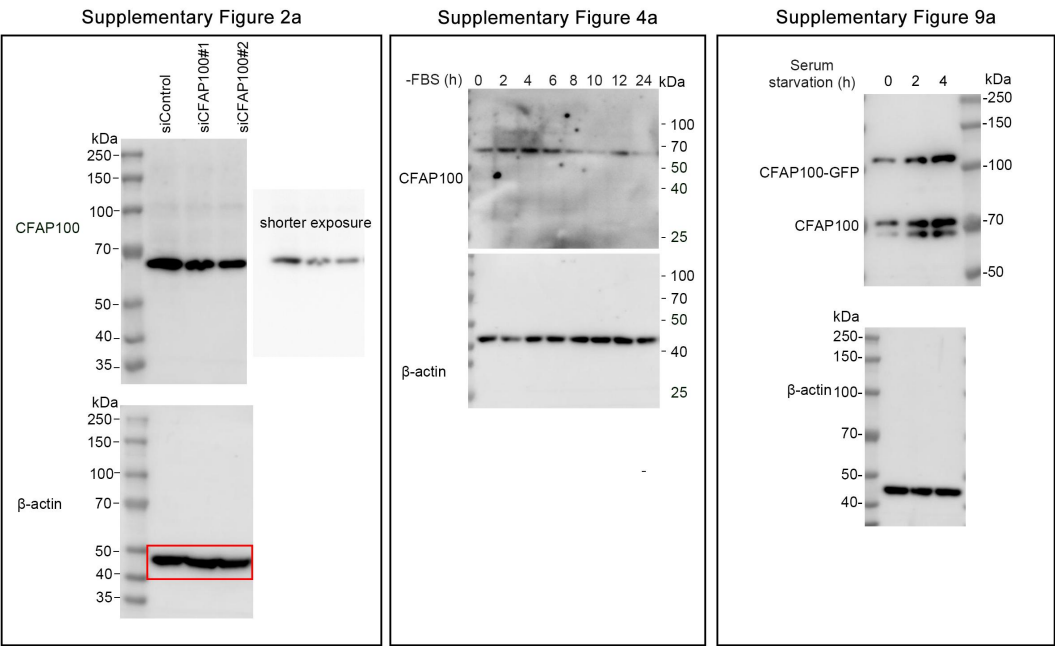


Supplementary Figure 12 Analysis of the properties of CFAP100 mutants. a Quantification of droplet formation in RPE1 cells transfected with GFP, CFAP100 FL-GFP, and the indicated mutants. **b** Immunoblot analysis of WT and *CFAP100* KO RPE1 cells stably expressing CFAP100 FL-GFP and the indicated mutants. The levels of CFAP100-GFP expression are comparable to that of endogenous CFAP100. **c** Quantification of ciliary length in WT and *CFAP100* KO RPE1 cells infected with lentiviruses expressing GFP, CFAP100-GFP, or the indicated mutants, and cultured in serum-free medium for 4 h. $n = 200$ cilia from 3 independent experiments. Data are presented as mean $\pm$ SEM. P values were determined by one-way ANOVA with Dunnett's post-hoc analysis (**a**, **c**). Source data are provided as a Source Data file.

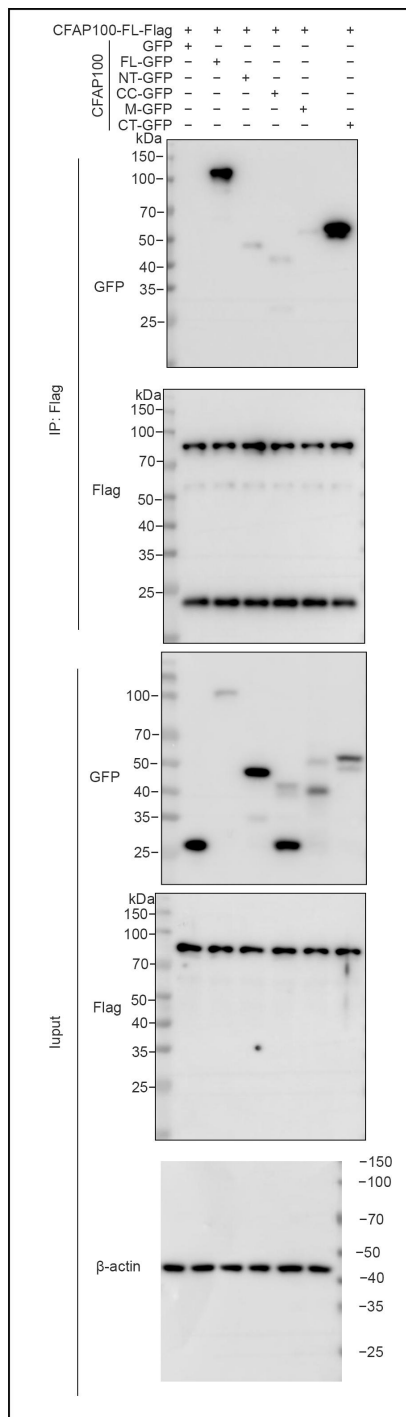


Supplementary Figure 13 **NPHP3 depletion does not significantly affect ciliogenesis.** **a-b** Immunoblot analysis of NPHP3 expression (**a**) and quantification of its relative level (**b**, $n = 3$ independent experiments) in RPE1 cells transfected with control or NPHP3-specific siRNAs and serum-starved for 48 h. **c-e**. Immunofluorescence microscopy (**c**) and quantification of the percentage of ciliated cells (**d**, $n = 3$ independent experiments) and ciliary length (**e**, $n = 300$ cilia from 3 independent experiments) for MEFs cultured in serum-free medium for 48 h and stained with antibodies against ace-tubulin and CEP164 and DAPI. Scale bar, 10 μm. Data are presented as mean ± SEM. P values were determined one-way ANOVA with Dunnett's post-hoc analysis (**b**, **d**, and **e**). Source data are provided as a Source Data file.

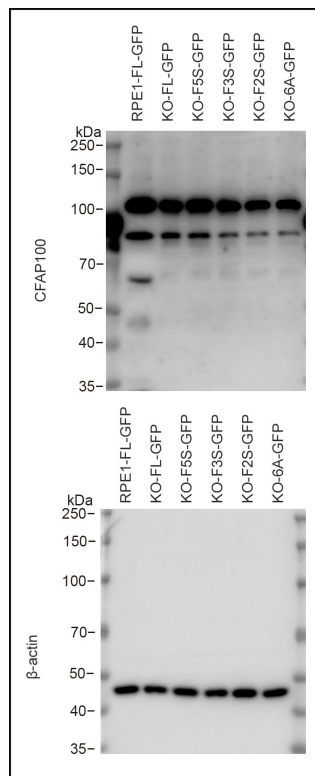
Uncropped scans of all blots



Supplementary Figure 11



Supplementary Figure 12b



Supplementary Figure 13a

