JCB

Ogungbenro et al., https://doi.org/10.1083/jcb.201706095



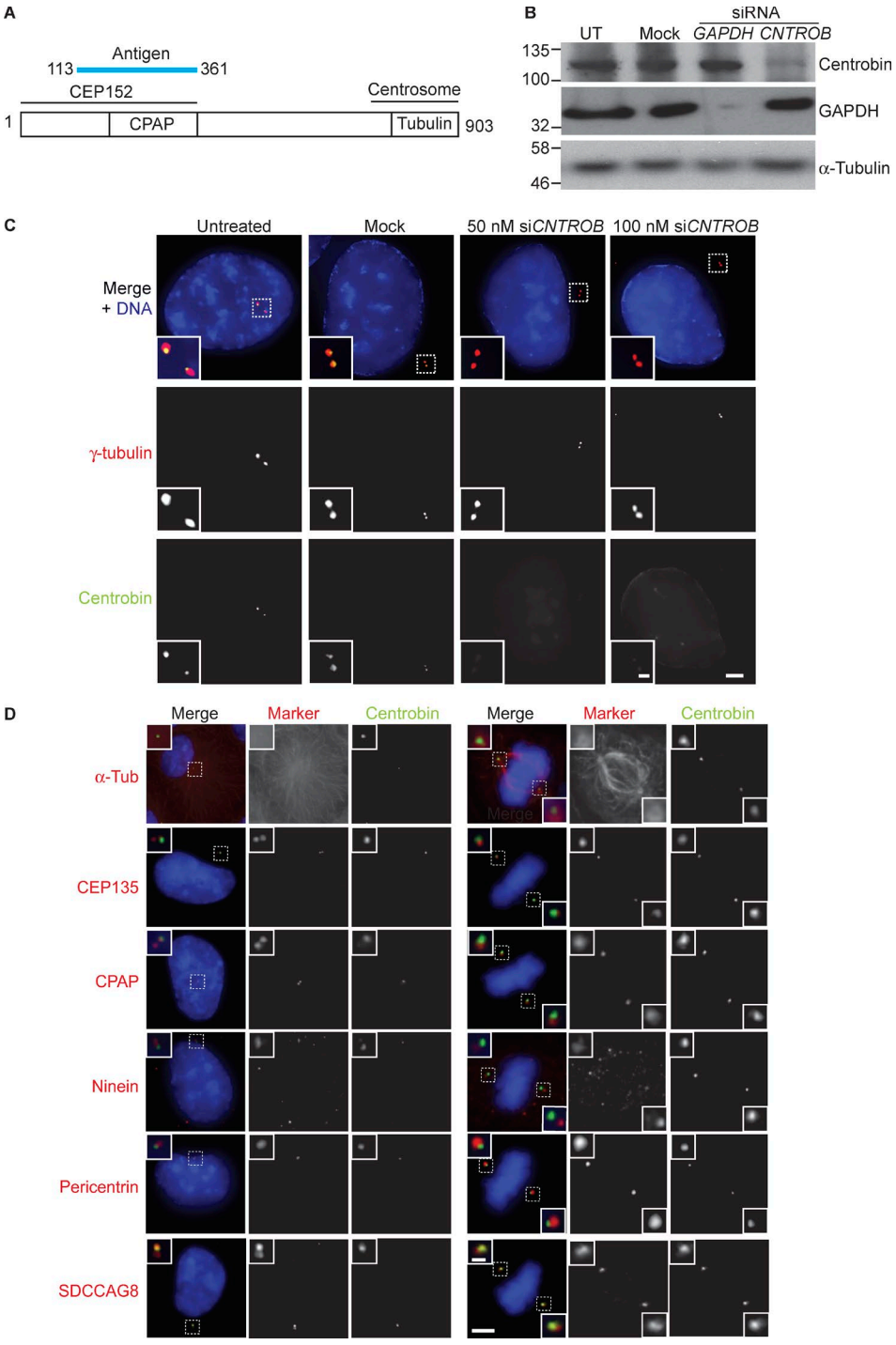


Figure S1. **Generation and characterization of a monoclonal antibody for centrobin. (A)** Diagram of centrobin indicating fragment of the protein used for generation of the monoclonal 6D4F4 and the regions required for CPAP, CEP152, or tubulin interactions or localization to the centrosome. **(B)** RNAi confirmation of the specificity of 6D4F4 using immunoblot analysis of control hTERT-RPE1 cells or of *CNTROB* knockdown cells 48 h after siRNA transfection with the indicated siRNA. UT, untreated. **(C)** Micrograph confirmation of the specificity of 6D4F4. *CNTROB* knockdown or control hTERT-RPE1 cells were stained for centrobin (green) and γ-tubulin (red) 48 h after siRNA transfection. Bars: 5 µm; (inset) 1 µm. **(D)** IF localization of centrobin. Interphase or mitotic cells were stained with the monoclonal antibody 6D4F4 to centrobin (green) and the indicated markers (red). Bars: 5 µm; (inset) 1 µm.

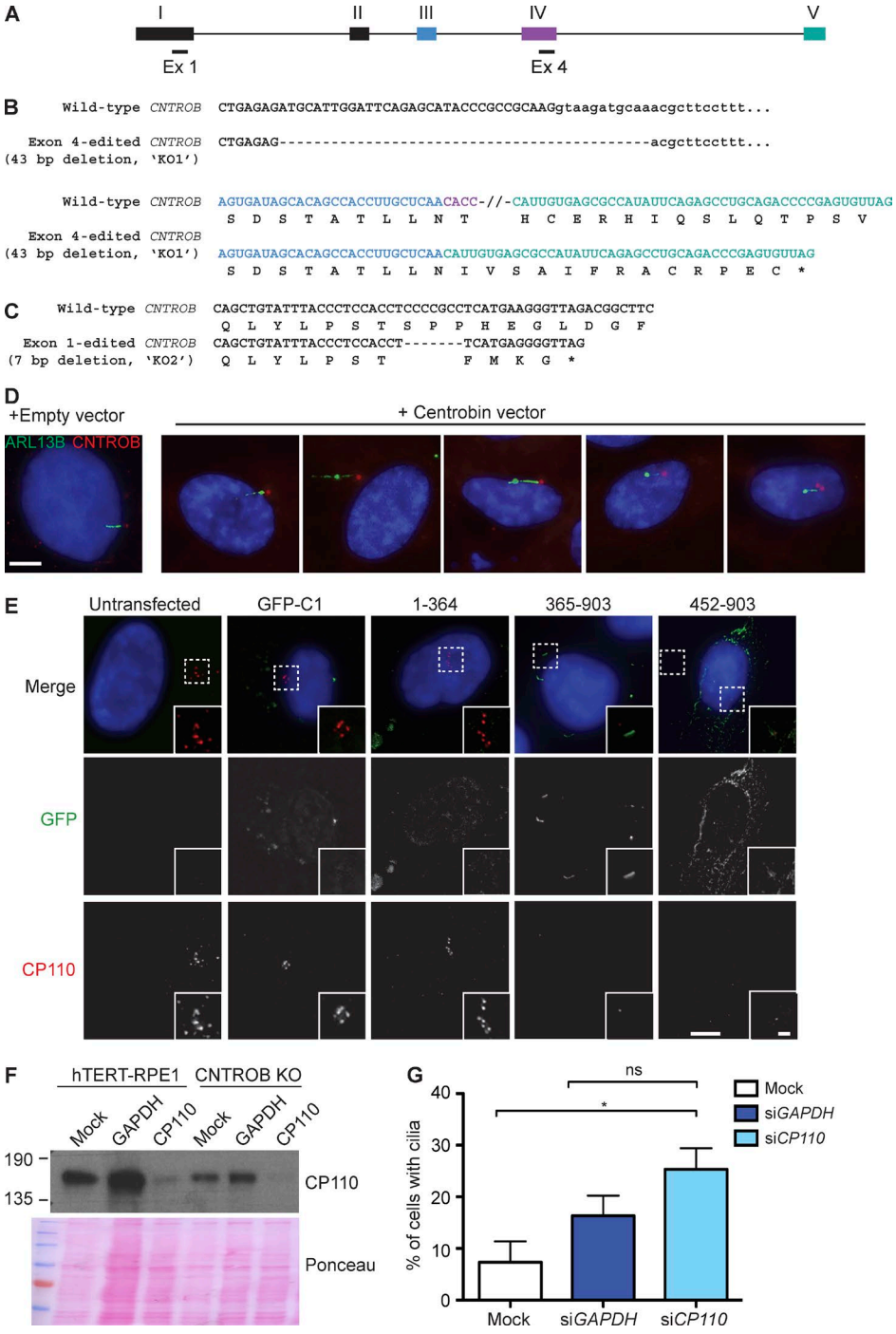


Figure S2. **Confirmation of *CNTROB* disruption by genome editing. (A)** Diagram of exons 1–5 of the 19 exons in the *CNTROB* locus showing the location of the guide RNAs used for CRISPR-directed genome editing (Ex 1 and Ex 4; not to scale). Exons are based on sequence NM_053051.3 and are indicated in color to clarify splicing in B. **(B)** Genome editing of *CNTROB* genomic sequence with the resultant transcript and predicted protein sequence for KO1 (exon 4 guide). Sequences are colored according to their respective exon as in A, and part of the WT exon 4 sequence is omitted. The 43-bp deletion at the 3′ end of exon 4 in KO1 led to splicing between directly exons 3 and 5, as indicated. **(C)** Genome editing of *CNTROB* genomic sequence with the resultant transcript and predicted protein sequence for KO2 (exon 1 guide). **(D)** IF micrographs of cells 48 h after transfection with the indicated plasmid. Cells were fixed and stained with antibodies to centrobin (red) and ARL13B (green), then counterstained for DNA with DAPI (blue). 35% of 100 cells transfected with the centrobin plasmid showed the "bulge," which was seen in only 4% of the control transfectants. Bar, 5 µm. **(E)** IF micrographs of *CNTROB* null cells after 24 h serum starvation imposed 16 h after transfection with the indicated GFP control or GFP-tagged centrobin fragment. Cells were fixed and stained with antibodies to CP110 (red) and GFP (green), then counterstained for DNA with DAPI (blue). Bars: 5 µm; (inset) 1 µm. Images are representative of two experiments in which CP110 accumulation was seen in a mean 65% of CNTROB knockout cells; 59% of the GFP-transfected cells; 65% of cells transfected with 1–364; 25% with 365–903; and 22% with 452–903. **(F)** Immunoblot showing the knockdown of CP110 in cells treated as indicated. Ponceau staining of the membrane after transfer was used as a loading control. **(G)** Bar chart shows quantitation of the ciliation frequency in *CNTROB* null cells after 48 h serum starvation with mock transfection, *CP110* knockdown and control *GAPDH* knockdown. Histogram shows mean + SEM of three independent experiments in which at least 100 cells were quantitated for ciliation by staining for acetylated tubulin. *, $P < 0.05$, in comparison to the indicated samples by unpaired *t* test.

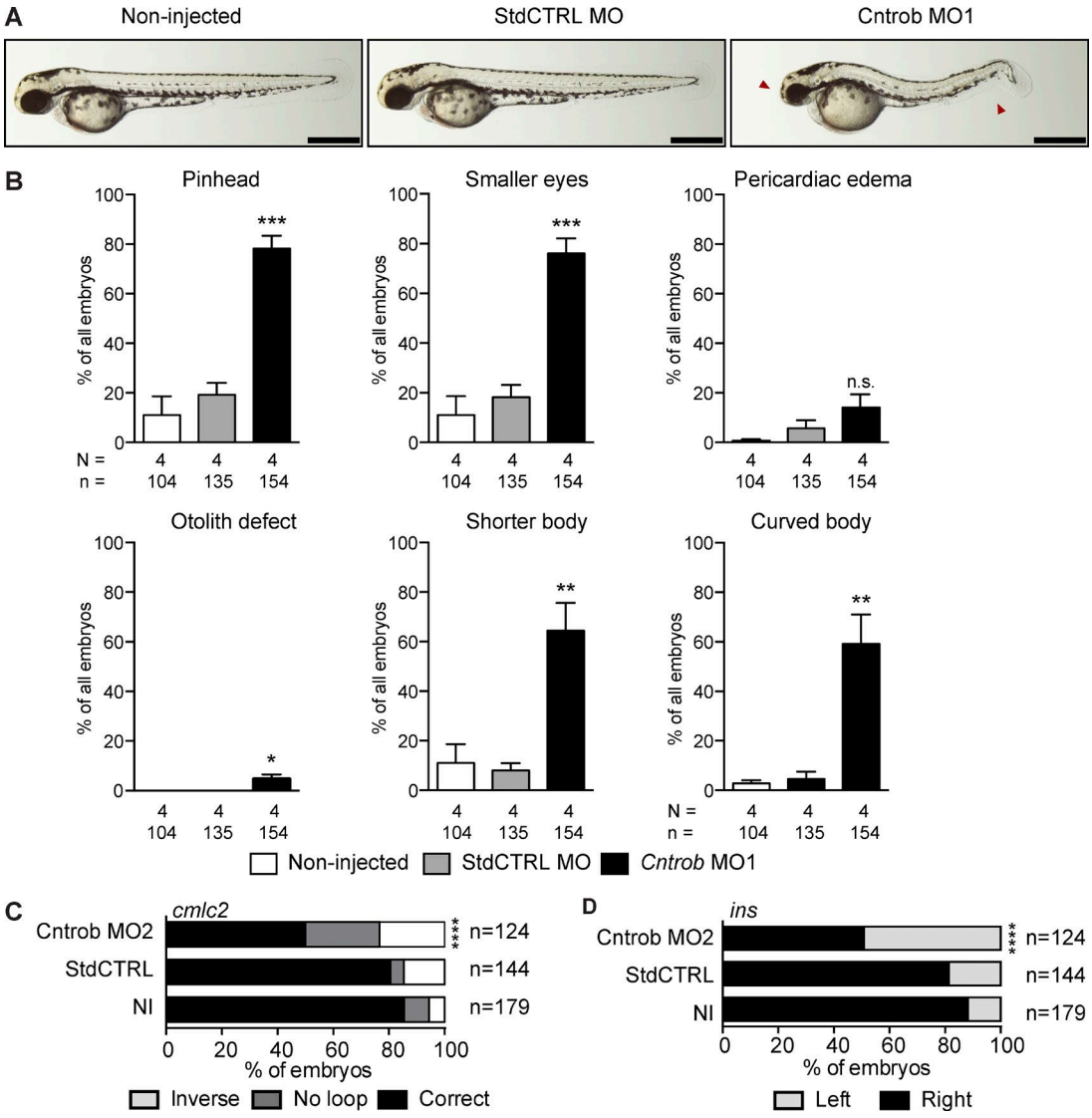


Figure S3. **Reproduction of the zebrafish phenotypes with a second MO.** MO1 was directed to the *centrobin* ATG and MO2 to the 5′ UTR. **(A)** Live images show gross phenotypes of zebrafish embryos injected with control or *centrobin* MOs at 48 hpf. Arrowheads indicate morphological abnormalities. Bars, 500 nm. **(B)** Quantitation of developmental phenotypes in centrobin-deficient embryos. Each phenotype was quantitated over three experiments in the indicated number of zebrafish embryos, and graphs indicate means + SEM. *, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$, by one-way ANOVA. **(C)** Quantitation of appropriate heart looping in WT embryos and embryos injected as indicated at 48 hpf. ****, $P < 0.0001$. Significances were assessed using Fisher's exact test. **(D)** Quantitation of pancreas placement in WT embryos and embryos injected as indicated at 48 hpf. Correct placement is at right. ****, $P < 0.0001$. Significances were assessed using Fisher's exact test.