Expanded View Figures

Figure EV1. Ccdc108 is essential for multiciliogenesis in epidermis of the *Xenopus* embryo (related to Fig 1).

A *In vivo* validation of morpholino efficiency. *ccdc108* morpholino inhibits the expression of the GFP reporter containing the MO target site of *ccdc108* at the 5'UTR.

B Scanning electron microscope images and graphical plot show slightly shorter cilia in Ccdc108-depleted MCCs. Experiment was performed once and 50 cilia from 10 MCCs in different microscopic fields were scored. Mean ± s.d. values are presented.

C Representative confocal images and graphical plot display reduced ciliary Ac-tub levels in *ccdc108* CRISPR mutants. A total of 20 images of 20 embryos for each condition. Cell membranes (mGFP, purple) and cilia (Ac-tub, green) were labeled with indicated antibodies. Quantitative data from three independent experiments were scored. Unpaired two-tailed *t*-test was performed (***$P < 0.001$). Mean ± s.d. values are also presented.

D Graphical plot and cartoons show that epidermal MCC cilia beat frequency (CBF) and cilia beat pattern (CBP) are both affected in *ccdc108* morpholino-treated *Xenopus* embryos from stage 27. The cartoons were generated based on the results of imaging live MCCs by high-speed video microscopy (Movies EV4–6). Two biologically independent experiments were performed. Greater than 15 MCCs from four embryos for each condition.

E Transverse section views show that Ccdc108 is dispensable for primary ciliogenesis in the *Xenopus* neural tube. Embryos at stage 30 were fixed, and stained with the acetylated tubulin antibody (red) and DAPI (blue). mRNA of a membrane-bound form of GFP (mGFP; green) was co-injected with each morpholino to indicate targeted cells. Arrows mark primary cilia. Three biologically independent experiments were performed and images from the same experiment were presented.

F Effects of Ccdc108 depletion on motile monocilia formation and length in the gastrocoel roof plate (GRP). Embryos were fixed and stained with the acetylated tubulin antibody (red). mGFP (green) was co-injected with each morpholino to indicate targeted cells. GRP explants were prepared from embryos at stage 18. Greater than 6 embryos from three biologically independent experiments for each condition were images and 10 cilia from each embryo were scored. Unpaired two-tailed *t*-test was performed. Mean ± s.d. values are also presented.

Source data are available online for this figure.

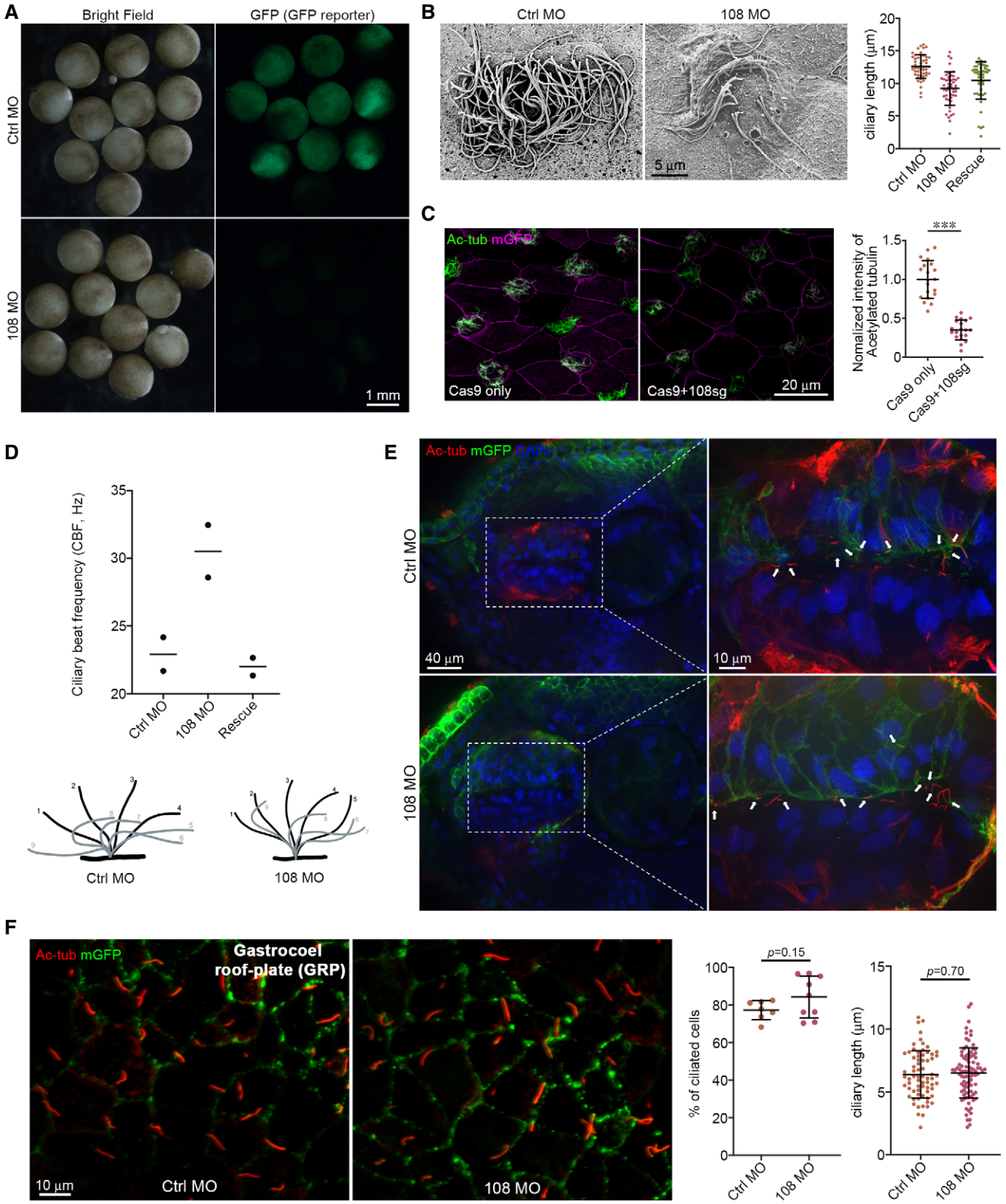

Figure EV1.

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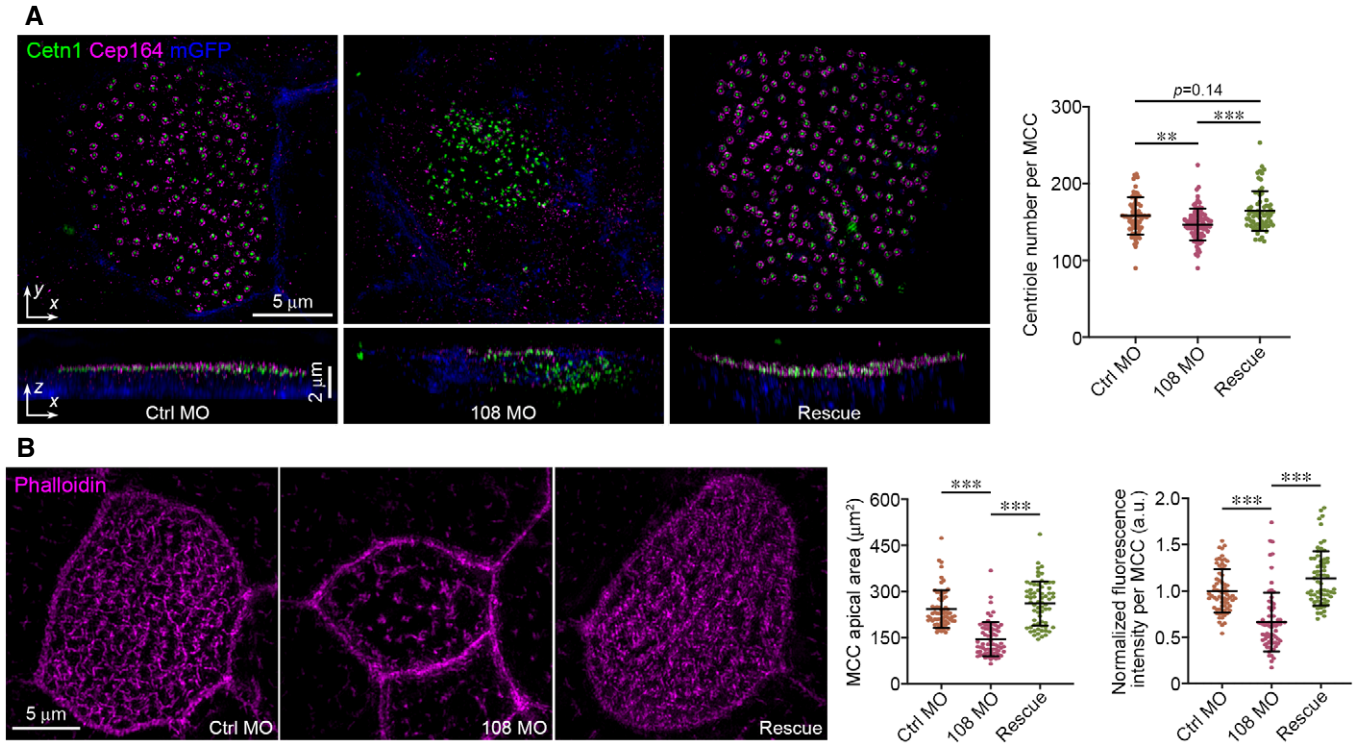

Figure EV2. Ccdc108 is required for apical trafficking of basal bodies and F-actin enrichment in MCCs of the *Xenopus* epidermis (related to Fig 3).

A Ccdc108 depletion affects centriole migration/docking to the apical surface in MCCs. Representative 3D-SIM images (*x*–*y*) and 3D reconstructions (*x*–*z*) of MCCs of embryos at stage 32 show a failure of apical trafficking of basal bodies in Ccdc108-depleted cells. Cell membranes (mGFP, blue), basal bodies (Cetn1, green), and distal appendages (Cep164, purple) were labeled with indicated antibodies. Greater than 60 MCCs from six embryos for each condition were counted.

B Representative 3D-SIM images show a significant reduction in apical expansion in MCCs of embryos at stage 32. Embryos treated as in Fig 1C were fixed at stage 32 and stained with phalloidin (purple). Greater than 60 MCCs from six embryos for each condition.

Data information: Quantitative data were from three independent experiments. Unpaired two-tailed *t*-test was performed (***$P < 0.001$; **$P < 0.01$). Mean ± s.d. values are presented.

Source data are available online for this figure.

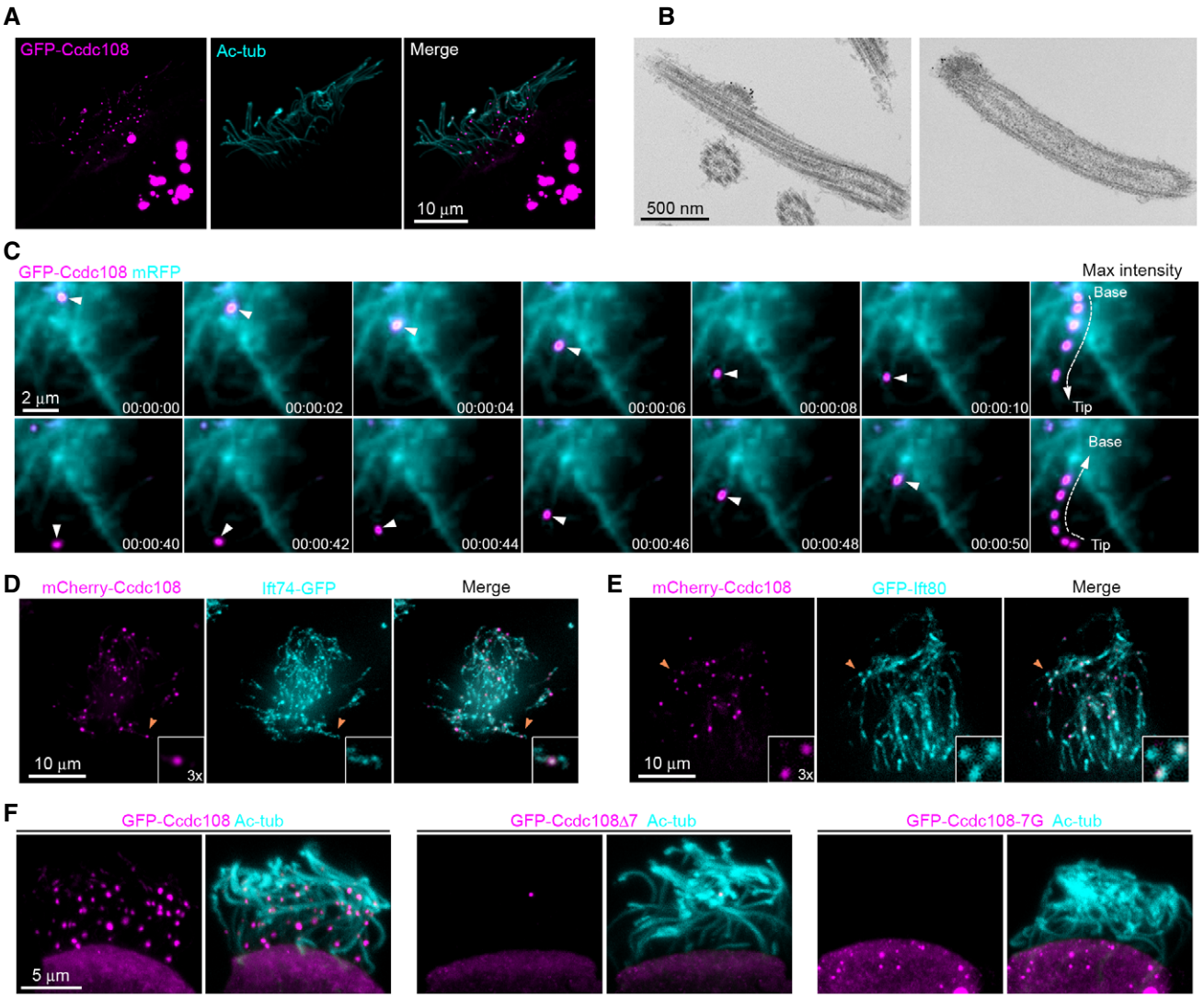

Figure EV3. Overexpression of Ccdc108 forms cytoplasmic granules and ciliary puncta along the axoneme in *Xenopus* epidermal MCCs (related to Fig 4).

A Representative confocal images show the distribution of Ccdc108 in MCCs cilia and cytoplasm. Embryos were injected with 500 pg mRNA of GFP-Ccdc108 (purple) at four-cell stage, fixed at stage 27 and labeled with the Ac-tub antibody (cyan).

B Immuno-EM demonstrates that Ccdc108 localizes to the IFT train-like structures along the axoneme. Embryos expressing GFP-tagged Ccdc108 were immune labeled with 10 nm gold particles.

C Live cell imaging shows that Ccdc108 displays a bidirectional movement along the axoneme (Movie EV7). A live embryo expressing GFP-Ccdc108 (purple) and membrane-bound RFP (blue) was imaged at 2 s intervals using a spinning disk confocal microscope.

D, E Representative confocal images show Ccdc108 puncta co-localize with IFT proteins at the ciliary axoneme in MCCs. Embryos expressing mCherry-Ccdc108 (purple) and Ift74-GFP (D), or GFP-Ift80 (E) were fixed and labeled with related epitope tag antibodies.

F Representative confocal images show the inability of Ccdc108 mutant proteins lacking the seven amino acid IFT interaction domain to localize to cilia. Embryos expressing GFP-Ccdc108 or mutant proteins (purple) were fixed and labeled with the acetylated tubulin antibody (Ac-tub, cyan).

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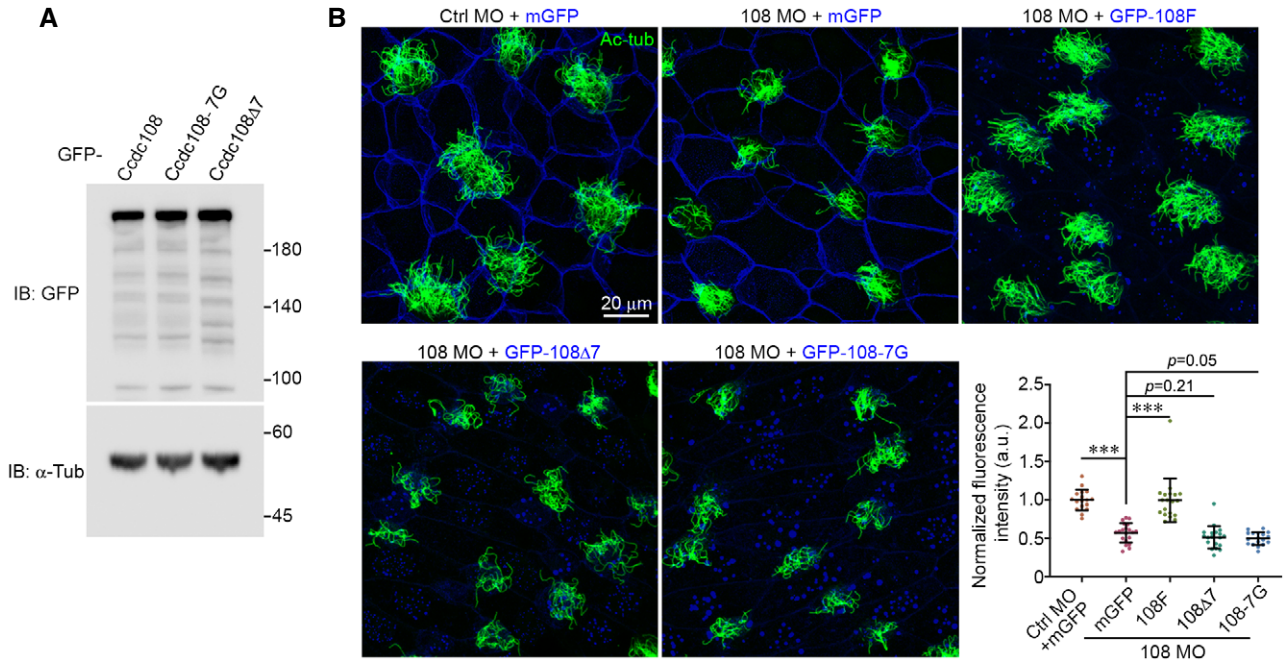

Figure EV4. The IFT interaction domain is required for the function of Ccdc108 in multiciliogenesis in *Xenopus* embryos (related to Fig 5).

A Immunoblotting reveals that wild-type and mutant Ccdc108 proteins display similar expression levels. *Xenopus* embryos expressing GFP-tagged wild-type or mutant Ccdc108 proteins were subjected to immunoblotting. α-tubulin (α-Tub) served as loading control.

B Representative confocal images show mutant Ccdc108 proteins fail to restore the impaired multiciliogenesis induced by Ccdc108 depletion. Embryos were treated as described in Fig 1C and expressed mGFP (blue) or GFP-Ccdc108 mutants (blue). Embryos were fixed at stage 27 and labeled with the Ac-tub antibody (green). Plots show results of greater than 17 images of 17 embryos from three independent experiments. Unpaired two-tailed *t*-test was performed (***$P < 0.001$). Mean ± s.d. values are presented.

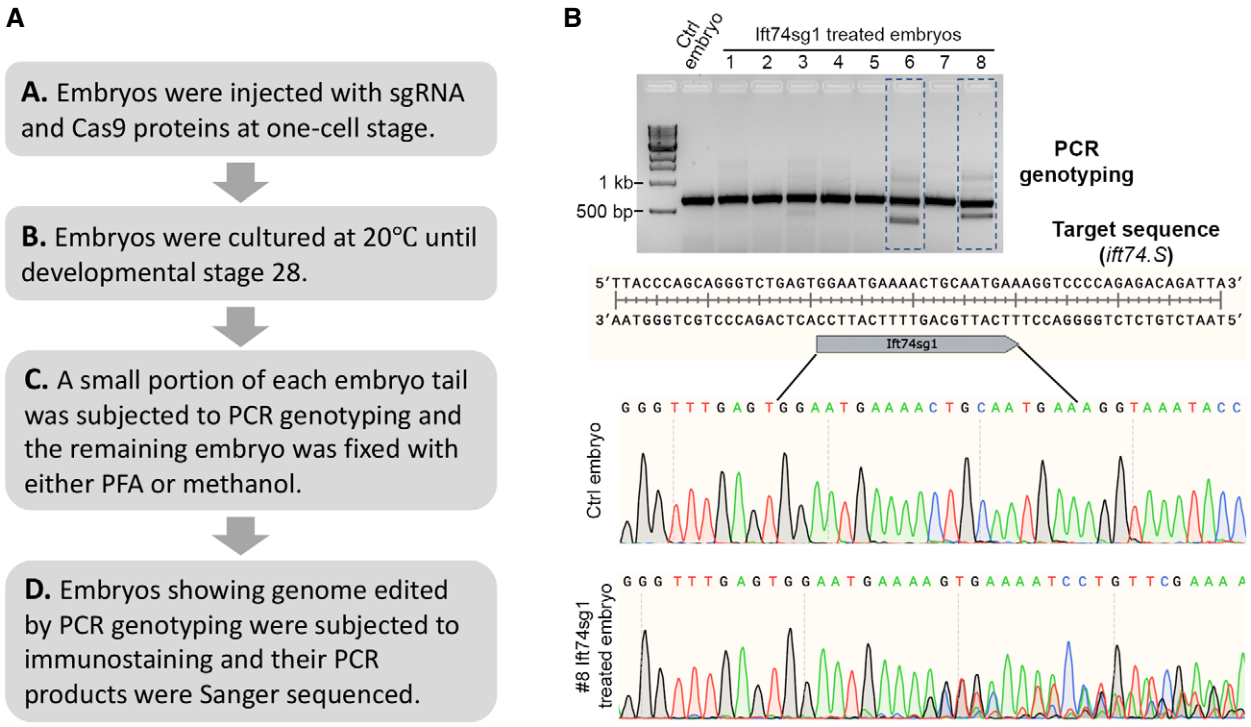

Figure EV5. ***In vitro*** **validation of CRISPR-Cas9-Mediated Genome Editing (related to Fig 6).**

A Experimental design of the *in vitro* validation.

B *In vitro* validation results of *Xenopus* Ift74sg1 used in this study. PCR primers were designed to produce a single-band PCR product that flanks the genomic edit of interest. The PCR products were resolved in 1.2% agarose gels stained with ethidium bromide. Gel image converted from electropherogram shows that two products (#6 and #8) display multiple bands compared to the control sample. Sequencing chromatograms show DNA editing of #6 PCR product. Underlined region indicates the sgRNA-binding sequence.