



Supplementary information, Figure S8 Disruption of NudC by CRISPR/Cas9 causes ciliary defects. One-cell embryos of zebrafish were co-injected Cas9 mRNA (250 pg) and NudC gRNA (20 pg) or not. (A) Cartoon showing the gene structure, the position of the target site and the restriction enzyme (Bcc I) cutting site (underlined) in zebrafish *NudC* locus. PAM (protospacer adjacent motif) site is indicated in red. (B) At 48 h after injection, 10 embryos were randomly selected and pooled to extract their genomic DNA for PCR amplification. PCR products were confirmed with restriction enzyme digestion and Sanger sequencing. Representative sequencing results are shown. Target sequence is underlined; PAM sites are shown in red; mutations are indicated in green. (C, D) Bright-field micrographs show that the zebrafish co-injected with Cas9 mRNA and NudC gRNA exhibit body curvature, pericardial edema (arrow) and hydrocephalus (arrowhead) at 72 hpf. Scale bar, 500 µm. (E-G) Immunofluorescence with anti-acetylated-tubulin antibody (green) at 8 ss reveals longer cilia in Cas9/NudC-gRNA-treated embryos compared to that of the control (E). Scale bar, 10 µm. Cilia length in KV is measured by Image J software (F). The average number of cilia per KV is also shown (G). Each bar represents mean ± SD of three independent experiments. n, sample size. *** $P < 0.001$; ns, not significant ($P > 0.05$).