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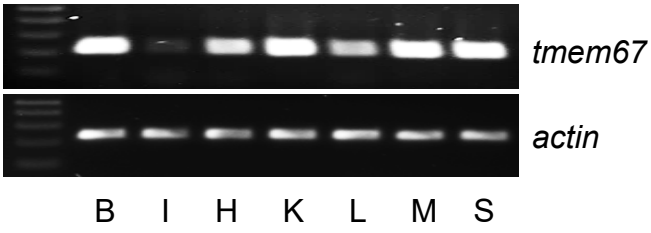
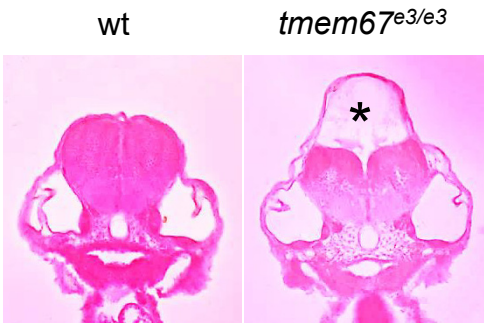
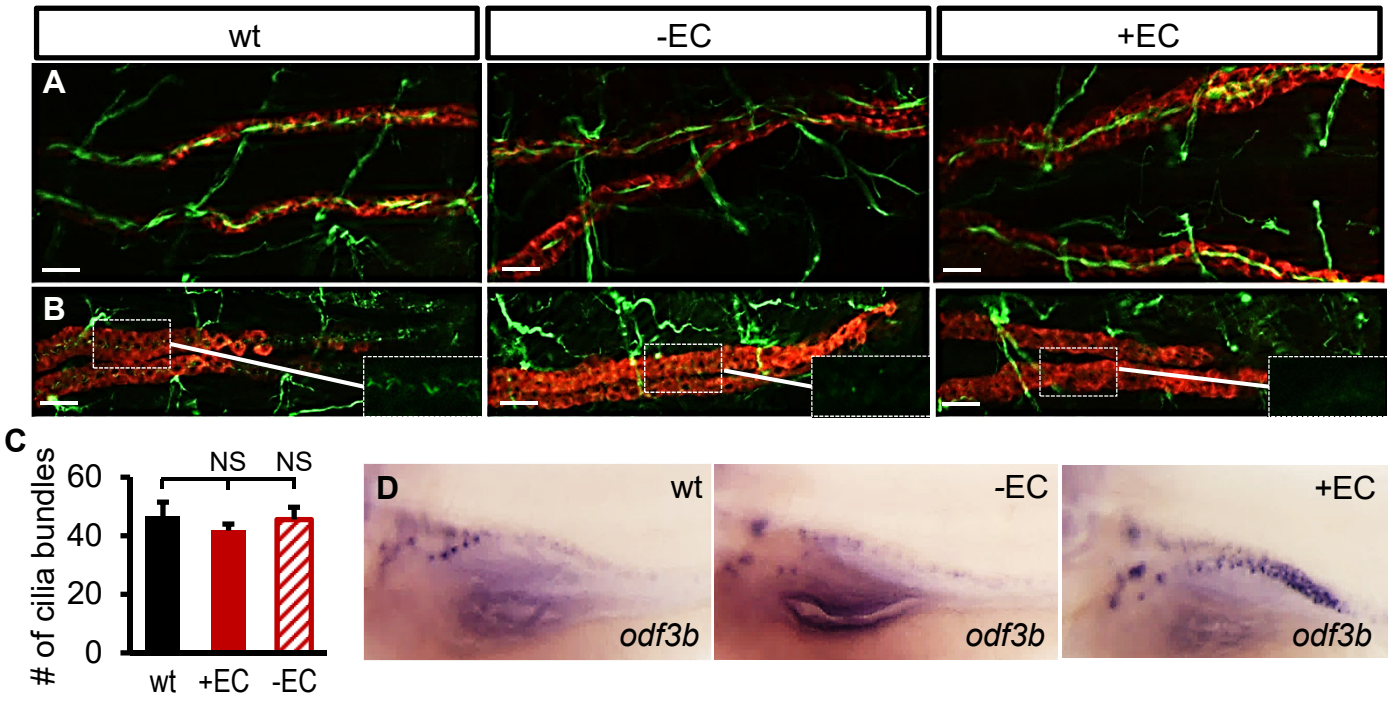


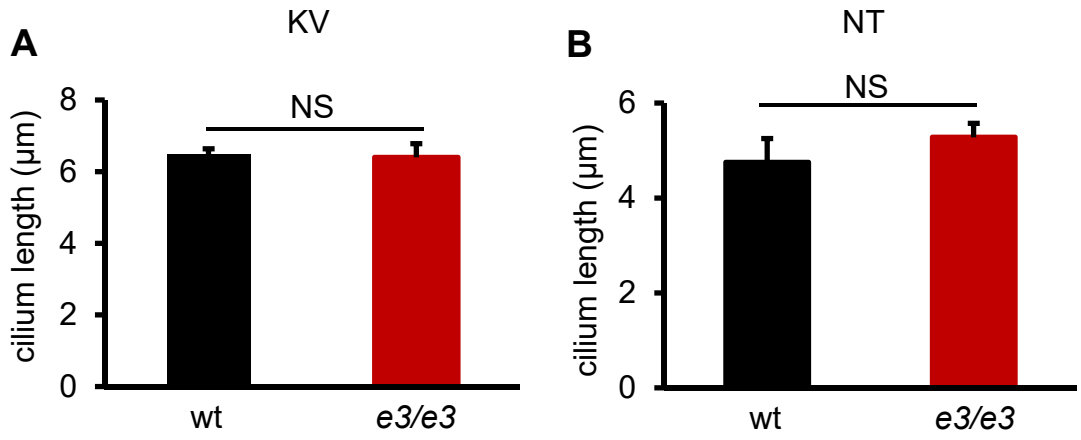
Supplemental Figure 1. *tmem67* is expressed in multiple tissues of the adult zebrafish
tmem67 transcripts were detected in the brain (B), heart (H), kidney (K), liver (L), muscle (M), spleen (S), and weakly in the intestine (I) via RT-PCR.



Supplemental Figure 2. Small percentage of *tmem67*$^{e3/e3}$ embryos develops hydrocephalous
Embryos at 3 dpf were assessed for the presence of hydrocephalous by HE staining. Three out of 85 *tmem67*$^{e3/e3}$ fish showed hydrocephalous (asterisks) while 0 out of 79 wild-type embryos did.

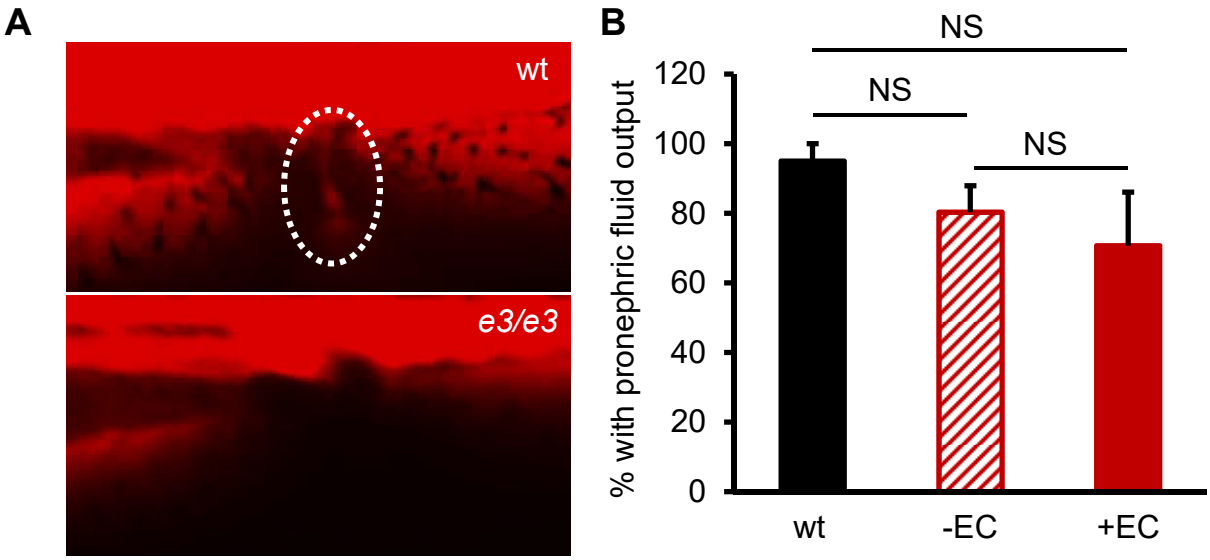


Supplemental Figure 3. Ciliary and MCC defects in *tmem67*$^{e3/e3}$ embryos at 4 dpf
(**A,B**) Multicilia bundles (A) and distal single cilia (B) of the pronephros were shown by co-immunostaining using antibodies against a-acetylated tubulin (green) and Na^+/K^+ ATPase (α6F, red). Enlargement of cilia staining in the boxed areas were shown in insets. **(C)** Quantification of multicilia bundles. (**D**) MCCs were indicated by *in situ* hybridization using riboprobe against *odf3b*. A total of 10-12 embryos per group per experiment were examined. Scale bar: 10 μm (A,B).



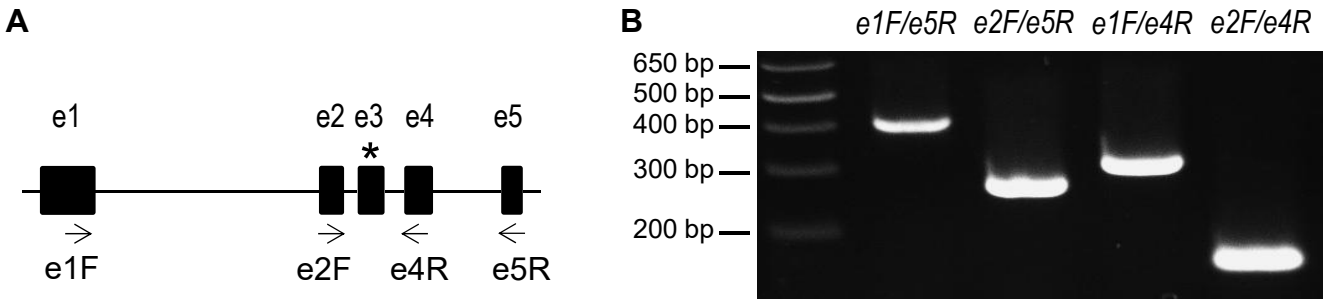
Supplemental Figure 4. Cilium lengths are not significantly altered in the Kupffer's vesicle and neural tubes

Embryos were collected at 10 somites (A) and 26 hpf (B) for cilia length analysis in the Kupffer's vesicle (KV, A) and neural tubes (NT, B), respectively. Eight to 12 embryos per group were analyzed. NS: not statistically significant.

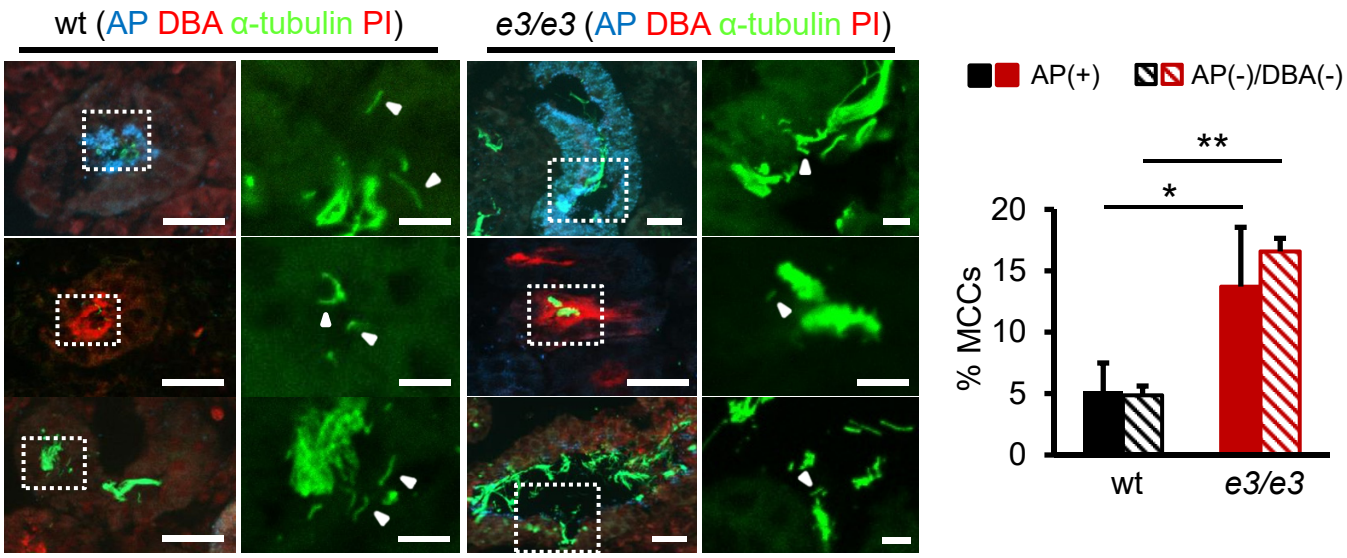


Supplemental Figure 5. Fluid excretion function of the kidney

(**A**) Embryo at 3 dpf was injected with rhodamine-dextran (10 kD)) via the common cardinal vein, inspected for the fluorescence dye at the cloaca (dashed circle) 30 minutes later, and then examined for pronephric cyst formation, followed by genotyping. (**B**) Quantification of pronephric fluid flow. Data are presented as means ± s.d. from three independent experiments. Seven to nineteen embryos per genotype were examined in each experiment. NS: not statistically significant ($P > 0.05$).

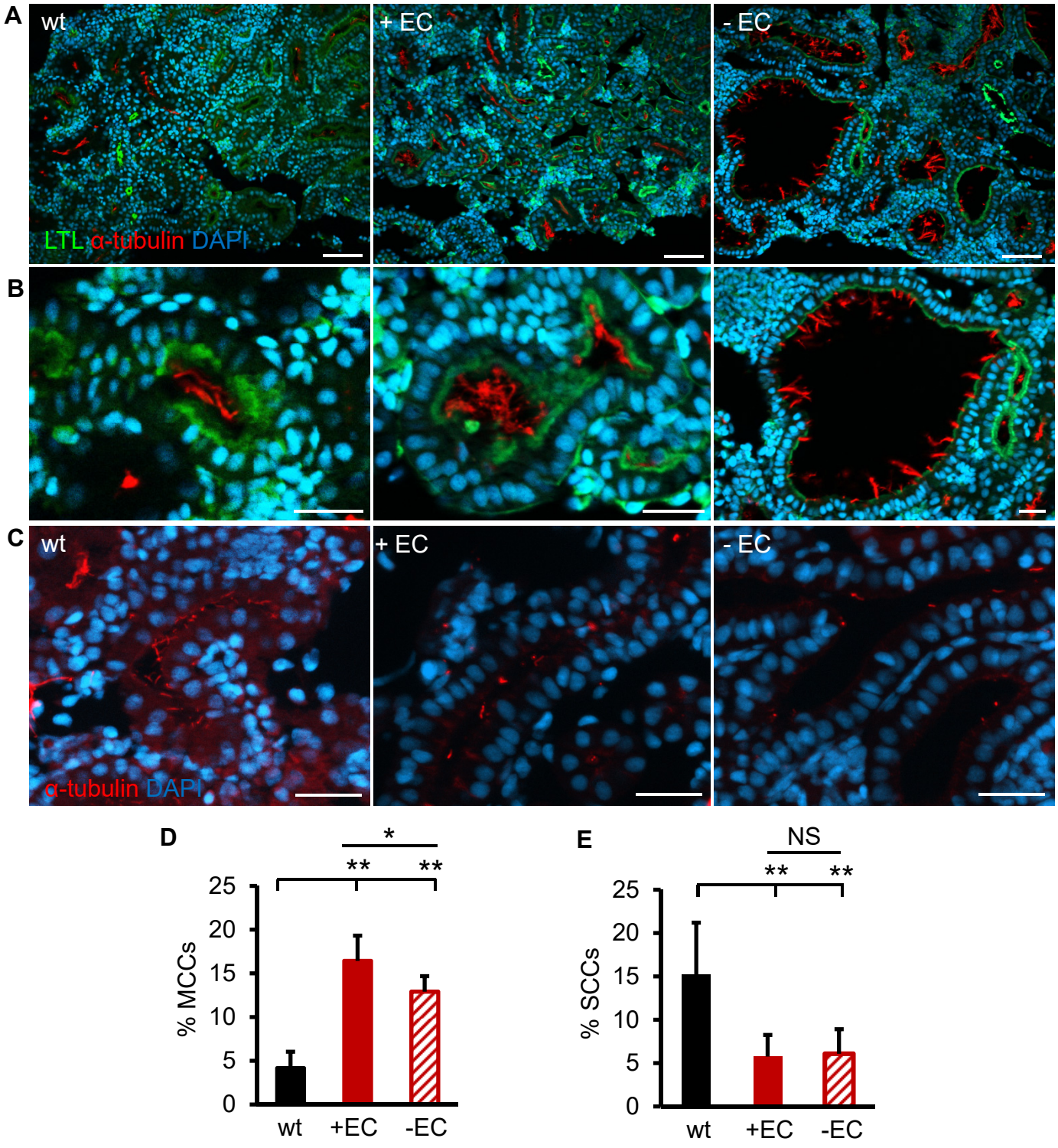


Supplemental Figure 6. *tmem67*$^{e3/e3}$ mutants do not show exon skipping events
(**A**) Schematic diagram showing exons surrounding the targeted exon3 of *tmem67* gene and primer binding sites. (**B**) RT-PCR amplification of cDNAs from the *tmem67*$^{e3/e3}$ embryos. Without alternative splicing events, PCR products are predicted to be 422 bp using e1F/e5R, 276 bp using e2F/e5R, 316 bp using e1F/e4R, and 170 bp using e2F/e4R, respectively. *tmem67-e1F*: TTCTCCATATCATTTCGACAGC; *tmem67-e2F*: GGTCAGTAATGGGGTGTCTATC; *tmem67-e4R*: AGGGATTGCCATTCCCATC; *tmem67-e5R*: GAGTTAATGAATGAGTCCCCAC.



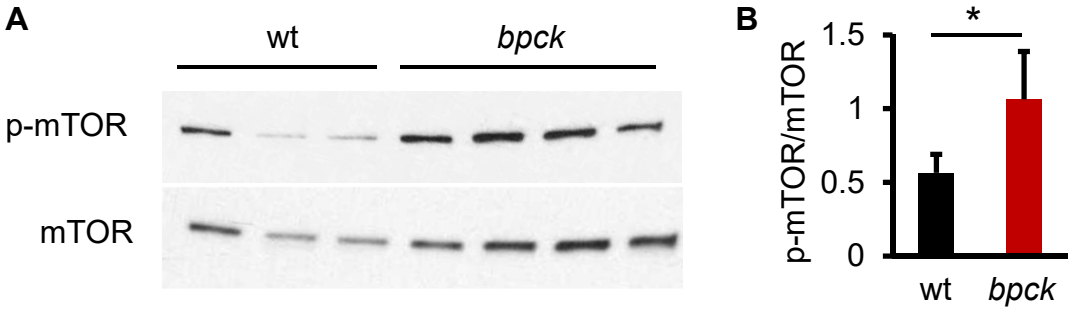
Supplemental Figure 7. Cilia abnormality in *tmem67*$^{e3/e3}$ adult zebrafish kidney

Cilia were examined by α-acetylated tubulin (green) antibody staining of frozen sections. The sections were colabeled with AP (blue), DBA (red), and trace amounts of Propidium Iodide (PI) (red; labels nuclei). Shown from left to right are merged images and cilia-only enlargement of the boxed area. The arrowhead indicates a single cilium. The percentages of MCCs were determined in the PT (AP(+)) and unstained (AP(-)/DBA(-)) tubules. The percentages of MCCs in the DTs cannot be quantified due to the narrow lumen. Four male fish per genotype were examined at 9 months. A total of 300-800 cells in each segment were counted. The data are presented as the mean ± s.d. *: $P<0.05$; **: $P<0.01$. Scale bars: 5 μm (L,M)

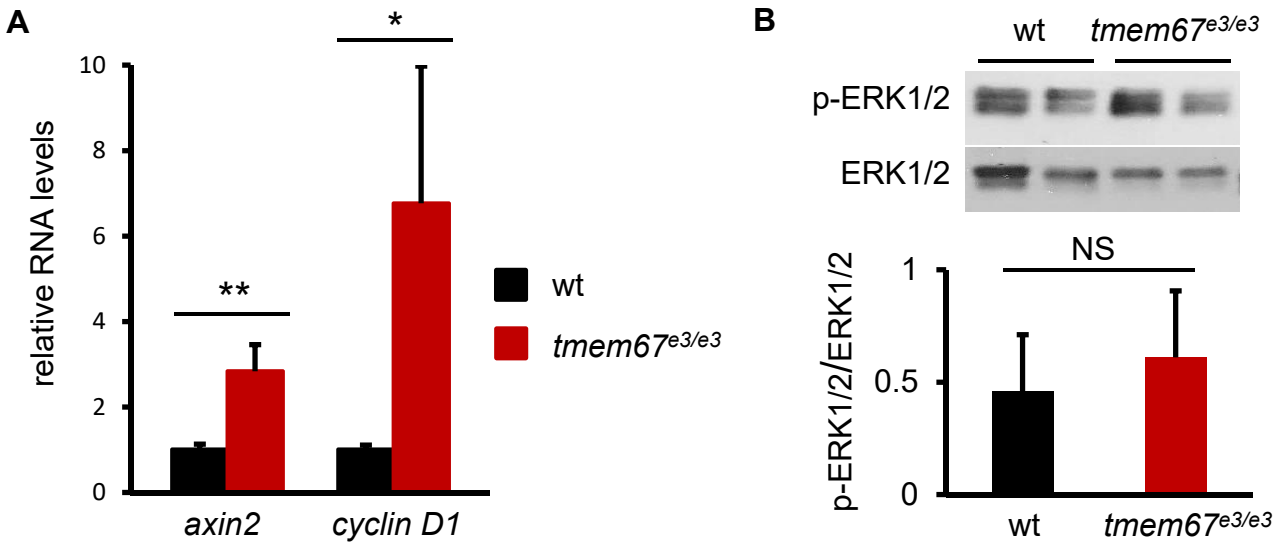


Supplemental Figure 8. Renal cilia in the +EC and –EC fish at 4 months

Immunostaining of paraffin sections was carried out using a α-acetylated tubulin (red) antibody, and the sections were costained with LTL (green) and DAPI (blue). (**A,B**) Cilia bundles were shown in LTL-tubules (A) and enlarged in (B). (**C**) To clearly present single cilia, LTL labeling was removed. (**D, E**) Quantification of the percentages of MCCs (D) and SCCs (E). Four fish per group and ~250 cells from MCC tubules or SCC tubules per kidney were examined. The data are presented as the mean ± s.d. *: $P<0.05$; **: $P<0.01$; NS: not statistically significant ($P>0.05$). Scale bar: 50 μm (A), 20 μm (B,C).



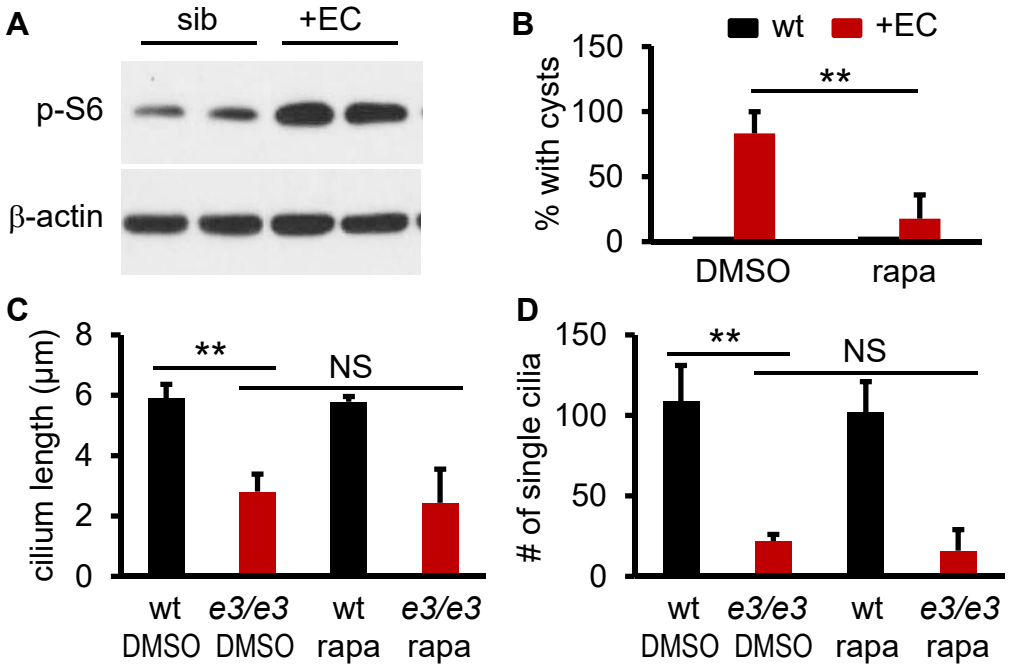
Supplemental Figure 9 . mTOR is activated in *bpck* mice
Kidneys from 3-week-old wild-type or *bpck* mice were collected. Phosphorylation pf mTOR was analyzed by western blotting (A) and quantified (B). *: $P<0.05$.



Supplemental Figure 10. The effect of *tmem67* disruption on Wnt/β-catenin and MAPK signaling

(**A**) Wnt/β-catenin target genes are overexpressed in *tmem67*$^{e3/e3}$ fish. Kidneys from 4 wild type siblings and 5 *tmem67*$^{e3/e3}$ fish were collected at 4 months for Q-PCR analysis. Primers used were described previously.[1-2] Gene expression was normalized by *gapdh*. (**B**) MAPK signaling appears not affected in *tmem67*$^{e3/e3}$ fish. Shown are representative immunoblots of three independent experiments. Total 12 kidneys per genotype were collected at 7-9 months. *: $P<0.05$. **: $P<0.01$. NS: not statistically significant.

1. Tuttle, A.M., Hoffman, T.L. & Schilling, T.F. Rabconnectin-3a regulates vesicle endocytosis and canonical Wnt signaling in zebrafish neural crest migration. *PLoS Biol* **12**, e1001852 (2014).
2. Miyake, A. *et al.* Neucrin, a novel secreted antagonist of canonical Wnt signaling, plays roles in developing neural tissues in zebrafish. *Mech Dev* **128**, 577-90 (2012).



Supplemental Figure 11. The effect of rapamycin on pronephric cyst formation and cilium biosynthesis in *tmem67*$^{e3/e3}$ embryos

(**A**) Phosphorylation of S6 protein was increased in +EC embryos at 5 dpf. Shown are representative images of three independent experiments. (**B**) Rapamycin treatment reversed pronephric cyst formation. +EC or wild-type embryos were incubated with rapamycin (400 nM) or DMSO at 56 hpf for 16 hours. The percentages of embryos with kidney cysts were analyzed by HE staining of JB-4 sections. (**C, D**) Rapamycin did not restore distal single cilium length and number. *e3/e3* and wild-type siblings were incubated with rapamycin at 56 hpf for 16 hours, and distal single cilia length and number were quantified following co-immunostaining using antibodies against a-acetylated tubulin and Na^+/K^+ ATPase (α6F). Twelve to 16 embryos per group were analyzed (B-D). **: $P<0.01$. NS: not statistically significant.