Supplementary Figures

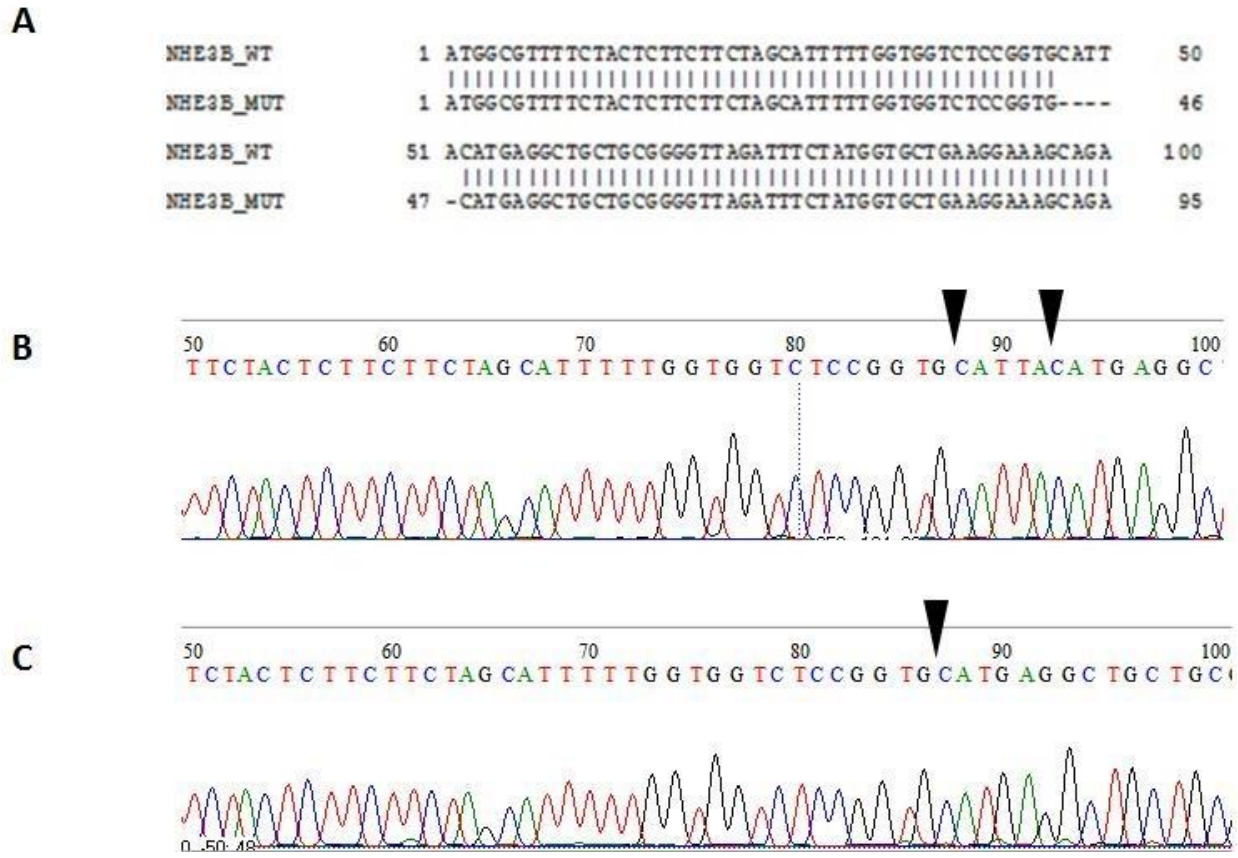

Figure S1. DNA sequence of a deletion mutation of the NHE3b transcript in zebrafish used in the study generated using the CRISPR-Cas9 gene editing technique. Sequence alignment of wildtype .and mutant genotypes displays a 5bp deletion in the mutants near the N-terminal of the transcript, between base pairs 46 - 51 of the wild-type transcript (A). Sanger sequencing data of DNA digests obtained from adult fin clips display the base pair differences between the wildtype (B) and mutant (C) genotypes, as indicated by black arrows.

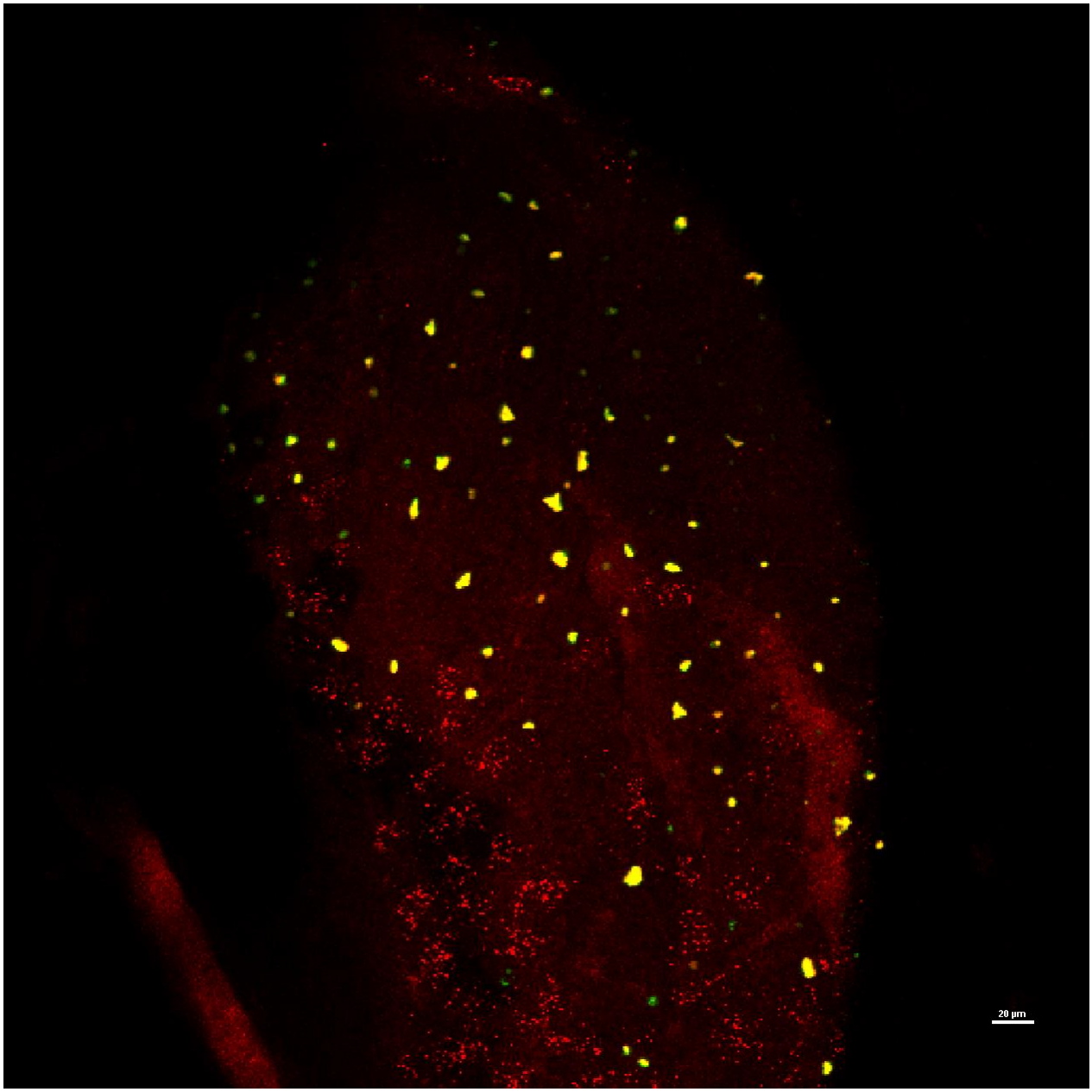

Figure S2. Representative whole mount immunohistochemistry images indicating the presence of (A) NHE3b (red) or (B) concanavalin A (ConA; green) in yolk sac epithelium of wild-type zebrafish (*Danio rerio*) larva at 4 dpf. The merged image (C) illustrates that NHE3b is co-localised to ConA positive cells indicating that these cells are HR cell ionocytes. A small percentage of ConA positive cells did not exhibit NHE3b staining. The scale bar represent 20 µM.

Methods**.** Larvae were exposed for 25 min to Concanavalin A (ConA) Alexa Fluor 633 conjugate (50 mg/ml), killed with anaesthetic overdose (MS-222) and fixed for 45 min with 4% PFA in PBS

(pH 7.3). After washing with PBS-T, specimens were blocked for 1 h using 3% BSA/PBS-T and then incubated with an affinity purified rabbit polyclonal antibody (1:200 dilution) raised against a cocktail of synthetic peptides corresponding to two regions of rainbow trout NHE3b (position 755–769: GDEDFEFSEGDSASG; 818–839: PSQRAQLRLPWTPSNLRRLAPL**)** (Zimmer et al., 2016). The longer of the two antigenic peptides shares 91% (19/21) amino acid identity with the corresponding zebrafish sequence. Subsequent washing, application of secondary antibody and microscopic imaging were performed as described in Materials and Methods.