**Table S1. Sequence of the DNA construct used to transcribe the *slc9a3.2* (*nhe3b*) and *rhcgb* single guide RNA (sgRNA), primer sequences used in the generation of the sgRNA DNA construct, and primer sequences used for mutant genotyping.** In the DNA construct, underlined nucleotides represent the T7 RNA polymerase promoter, bolded sequence represent the CRISPR/Cas9 target sequence (specific to either *slc9a3.2* or *rhcgb*), and italicized nucleotides represent the Cas9 binding sequence. The two template oligos formed the initial DNA template through partial complementary binding, and the forward and reverse amplification oligos amplified the template.

| **DNA Construct Sequence** | |
|---|---|
| GCGTAATACGACTCACTATA**NNNNNNNNNNNNNNNNNNN***GTTTTAGAGCTAGAA ATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGT GCTTT* | |
| **Primer Name** | **Sequence (5'-3')** |
| *slc9a3.2* template oligo | GCGTAATACGACTCACTATATGCATTACATGAGGCTGCTG GTTTTAGAGCTAGAAATAGC |
| *rhcgb* template oligo | GCGTAATACGACTCACTATAGGGCAACTGCTTCGGCTCCA GTTTTAGAGCTAGAAATAGC |
| Universal template oligo | AAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACT AGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAA |
| Forward amplification oligo | GCGTAATACGACTCACTATAG |
| Reverse amplification oligo | AAAGCACCGACTCGGTGCCAC |
| *slc9a3.2* forward sequencing primer | GAAGAACCTCCTGAAACACCAC |
| *slc9a3.2* reverse sequencing primer | TGATAGTGGCAGAATGACTGCT |
| *rhcgb* forward sequencing primer | TGTGGCACTTCTTGAAAGTGAT |
| *rhcgb* reverse sequencing primer | GCGGGTAAACTGAGTCTGATGT |

**Table S2. Primers used for real-time PCR.**

| Name | Sequence (5'-3') | Genbank Accession Number | Reference |
|---|---|---|---|
| *actb2* forward | TTACCACTTCACGC CGACTC | NM_131263.1 | Present study |
| *actb2* reverse | GTCACCTTCACCGTT CCAGT | | |
| *slc9a3.2* forward | TGCAGACAGCGCCT CTAGC | NM_001113479.1 | Kwong and Perry (2016) |
| *slc9a3.2* reverse | TGTGGCCTGTCTCTG TTTGC | | |
| *atp6v1aa* forward | GAGGAACCACTGCC ATTCCA | NM_201135.2 | Kwong and Perry (2016) |
| *atp6v1aa* reverse | CAACCCACATAAAT GATGACATCG | | |
| *asic4b* forward | GAACTTGACGTCGG GGTCTT | NM_214786.1 | Present study |
| *asic4b* reverse | ACCGGTTTCACATG AGGTCC | | |
| *slc12a10.2* forward | GCCCCCAAAGTTTT CCAGTT | NM_001045001.1 | Kwong and Perry (2016) |
| *slc12a10.2* reverse | TAAGCACGAAGAGG CTCCTTG | | |

**Table S3. Predicted amino acid sequences of nhe3b and rhcgb KO mutants based on Sanger sequencing.**

| Genotype | Predicted amino acid sequence |
|---|---|
| **nhe3b KO** | MAFSTLLLAFLVVSGA*STOP |
| **rhcgb KO** | MGNCFGFQGHLRPAKKHQHQTQFTRGVLRLAGVHDHTFRSVCAV*STOP |

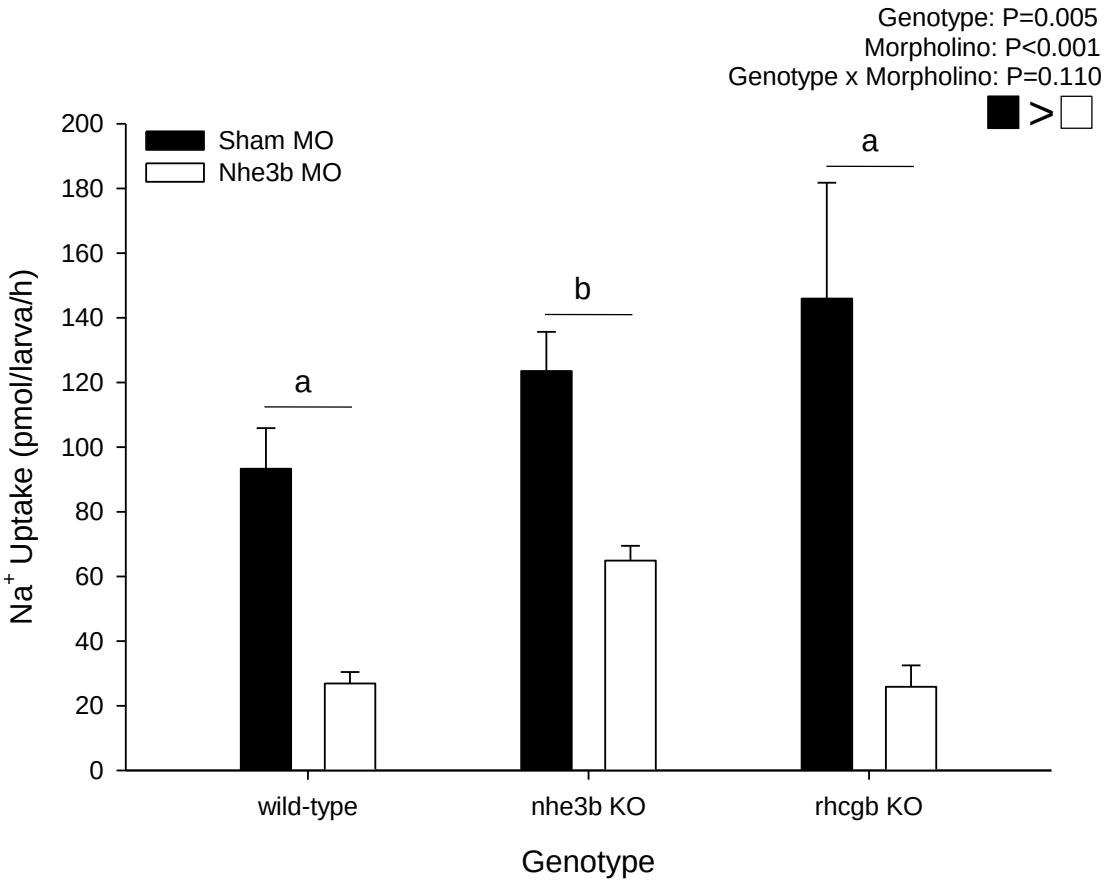


**Fig. S1. $Na^+$ uptake in response to a splice-blocking Nhe3b morpholino in wild-type, nhe3b KO, and rhcgb KO larvae.** $Na^+$ uptake rates in 4 dpf wild-type, nhe3b KO, and rhcgb KO larvae treated with sham (filled bars) or a splice-blocking Nhe3b morpholino (open bars; 5'-GCTCAGTGACTGGAAAGAGAAATA-3'; Kumai and Perry, 2011) morpholino (MO) and reared and assayed in 10 µmol/L $Na^+$. Letters above means that differ from one another represent a statistically significant effect of genotype within a morpholino treatment and boxes in the upper right corner of the panels depict the overall statistical effect of morpholino treatment as determined by a two-way ANOVA followed by a Holm-Sidak post-hoc test. (n=6-12)