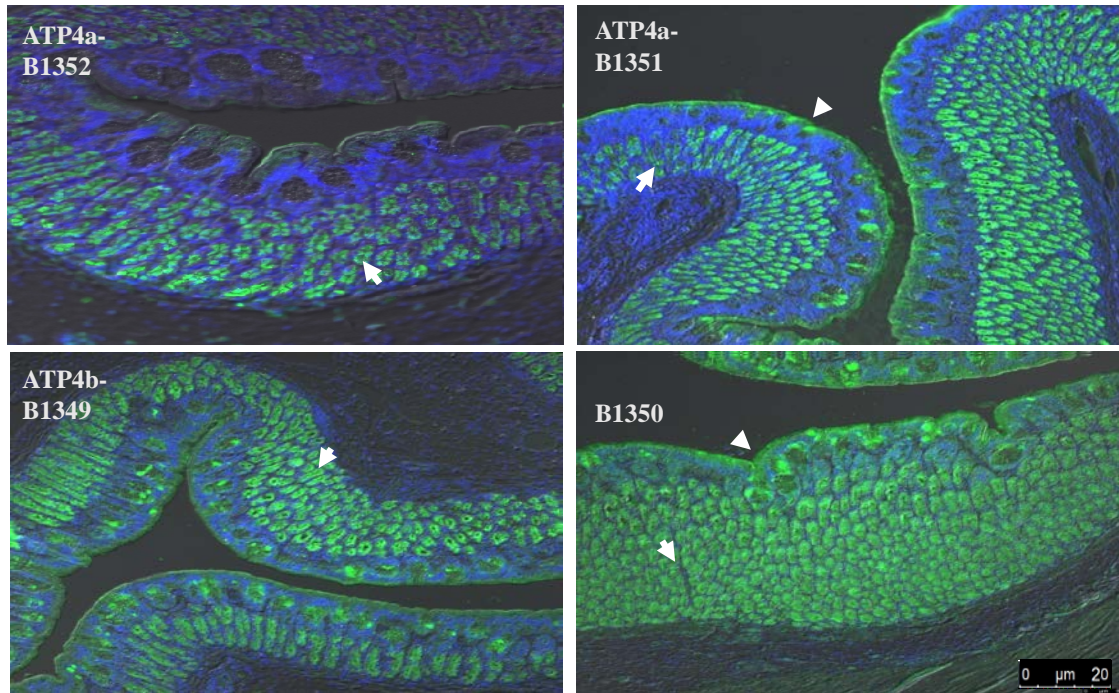


**Table S1.** Antibodies used for immunohistochemistry staining.

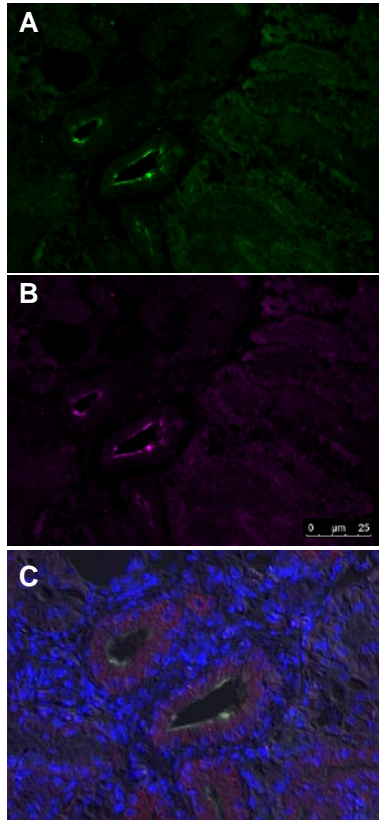
Target	Antibody/ Abbreviation	IHC Dilution	WB Dilution
Atp4a (HK $\alpha$ 1)	B1351	1:100-1:5000	1:1000-1:20000
Atp4a (HK $\alpha$ 1)	B1352	1:100-1:5000	1:1000-1:20000
Atp4b (HK $\beta$ )	B1349	1:100-1:5000	1:1000-1:20000
Atp4b (HK $\beta$ )	B1350	1:100-1:5000	1:1000-1:20000
Chicken pre-immune IgY	PI-IgY	1:100-1:5000	1:1000-1:20000
Normal rabbit serum	NRS	1:500	
Mouse negative control	J3 clone	1:100	
Atp1a1 (NK $\alpha$ 1)	$\alpha$ 5	1:100	
Atp1a1 (NK $\alpha$ 1)	$\alpha$ R1	1:500	

**Table S2.** RT-PCR primers designed using Primer3 prediction software and ensembl.org sequences for OrenilATP4A [ENSONIG00000005974](#) and OrenilATP4B [ENSONIG00000003180](#) used for determining intron sizes.

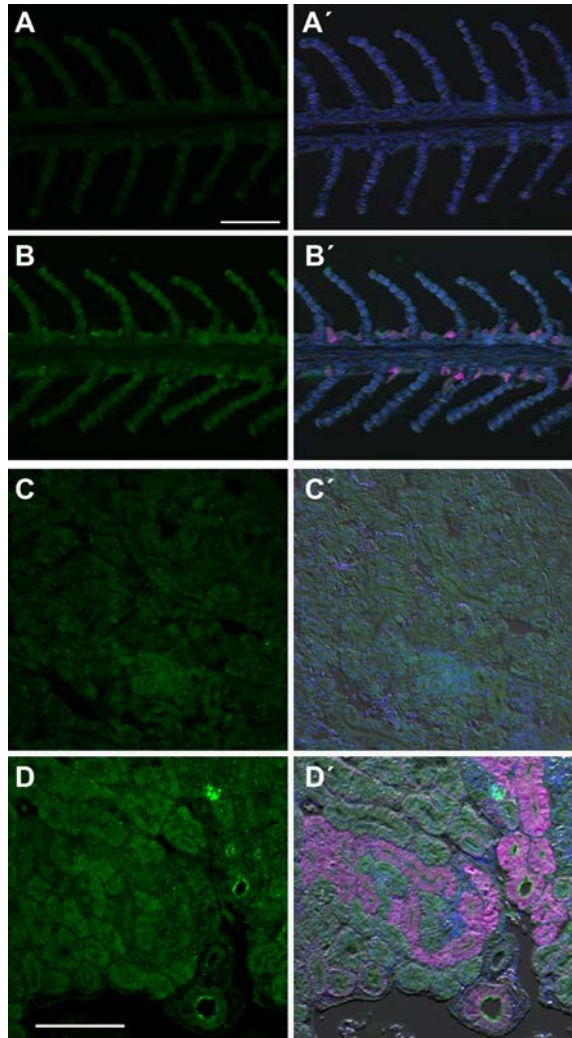
Target Gene (genbank)	5'-3' forward	5'-3' reverse
<b><i>atp4a</i> (XM_003450917.4)</b>	CATTCCAGCAAGGCTTCTTC	CACCAACTTCTGGGTGTCT
Product Size: 223 bp		
Introns: 436+232 = 668bp		
<b><i>atp4b</i> (XM_003445718.5)</b>	CTCGGAAAATGGGACGTTTA	GTCAGGGGTGTAGGGACTGA
Product Size: 148		
Intron: 1.2kb		
<b>18S (XR_003216133.1)</b>		
Product Size: 483	TCAAGAACGAAAGTCGGAGG	GGACATCTAAGGGCATCACA



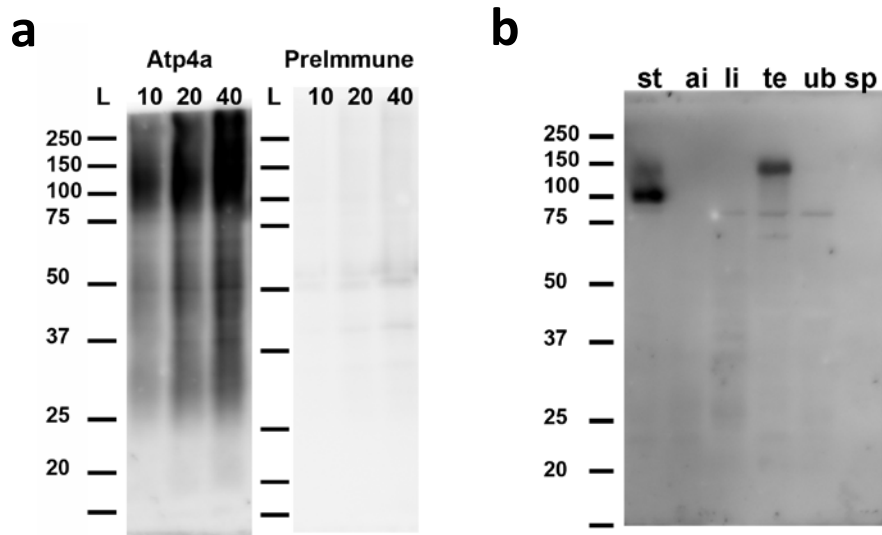
**Fig. S1** Immunohistochemistry of *O. niloticus* stomach with different antibodies for the gastric H<sup>+</sup>/K<sup>+</sup>-ATPase  $\alpha$  (Atp4a: B1351 and B1352) and  $\beta$  (Atp4b: B1349 and B1350) subunits showing a better specific staining for the gastric glands with Atp4a-B1352 and Atp4b-1349. Arrows indicate predicted gastric gland IR and arrowheads spurious IR with mucous columnar epithelial cells. Scale bar 20  $\mu\text{m}$ .



**Fig. S2.** Immunohistochemical localization of (A,C) Atp4a (Green), (B,C) Atp4b (Fuchia) and (C) NKA (Red) in the kidney collecting ducts of *O.niloticus*. (C) Sections are stained with DAPI for nuclei and the DIC image is overlaid. Scale bar 25 $\mu$ m.



**Figure S3. Negative IHC control sections.** (A-B) Gill sections were probed with either chicken (A,A') pre-immune IgY (Green) or (B,B') Atp4b-B1349 (Green) under identical conditions. Sections are double labelled with (A') normal rabbit serum and (B') NKA antibodies (Fuchsia), respectively. (C-D) Incubation of kidney sections with chicken pre-immune IgY (C,C') and Atp4b IgY (D,D'). Sections are double labelled with either NRS (C') or NKA  $\alpha$ R1 (D') antibodies. All sections (A',B',C',D') have nuclei counter stained with DAPI and the DIC image overlaid. Scale bars (A-B) 50  $\mu$ m, (C-D) 100  $\mu$ m.



**Figure S4. (a)** Immunoblotting of crude gill membrane extracts (10, 20, and 40 µg) from *O. niloticus* probed with Atp4a and Atp4b chicken IgY antibodies and preimmune IgY. **Figure S4(b).** Immunoblotting of expanded panel of crude tissue membrane extracts from *O. niloticus* probed with Atp4a chicken IgY antibodies. Organ panel (20 µg per lane): stomach (st), anterior intestine (ai), liver (li), testis (te), urinary bladder (ub) and spleen (sp). MW ladder: 250, 150, 100, 75, 50, 37, 25, 20 kDa and dye front.