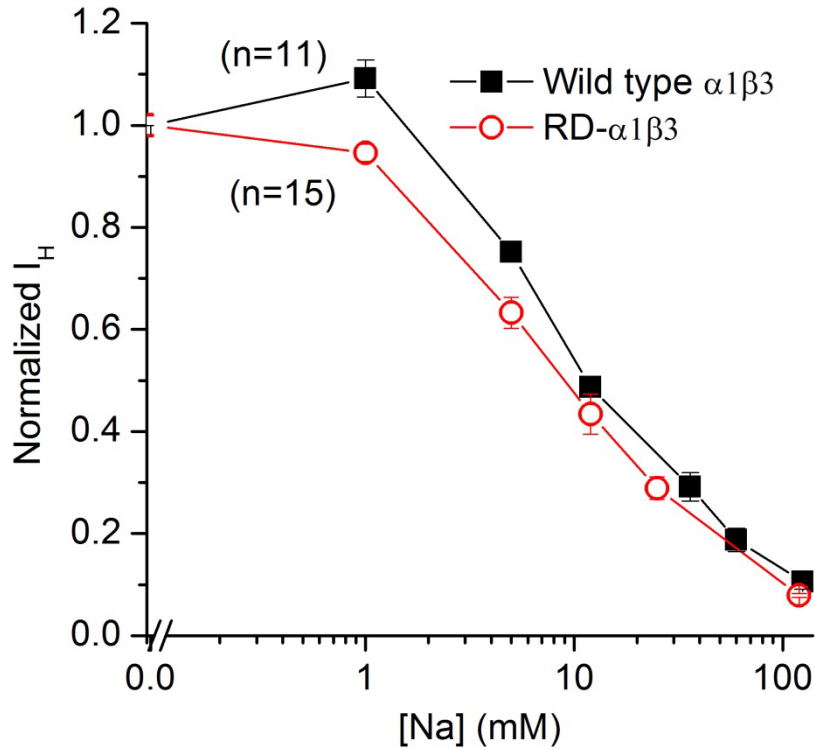
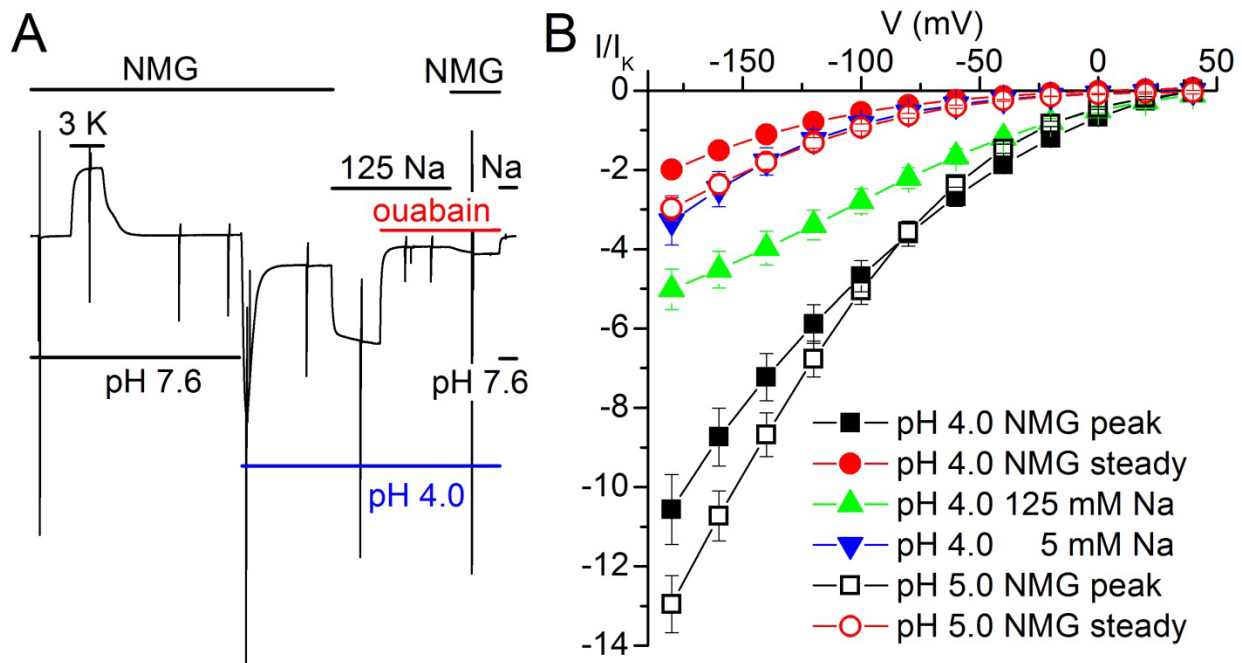


**Supplementary Fig. 1.** Peak and steady state currents at  $\text{pH}_o$  5.6 and 5.0. A) Ouabain-sensitive I-V curves measured at the peak of activation and after equilibration of the current at  $\text{pH}_o$  7.6, 5.0 and 5.6, normalized by  $I_H$  at -160 mV at pH 7.6. B) Ratio of the ouabain-sensitive steady state current and the current measured at the maximum activation by  $\text{H}^+$  was calculated for each individual oocyte and the mean  $\pm$  SE from the number of oocytes in parenthesis are shown. Inhibition by  $\text{H}^+$  is voltage independent. The data pH 4.0 (blue triangles) is from the experiments in Fig. S3.



**Supplementary Fig. 2.** Effect of  $\text{Na}^+$  on the  $I_H$  induced at -160 mV at pH 6. Averaged data from oocytes overexpressing wild type ouabain-sensitive  $\alpha 1\beta 3$  pumps (black squares) or RD- $\alpha 1\beta 3$  pumps (red circles). In the case of wild type pumps, application of ouabain irreversibly inhibited the current and thus we had to perform the changes in  $\text{Na}^+$  concentration before and after ouabain, making the experiment technically challenging as small changes in non-Na/K-pump-mediated currents could mask the actual effect of  $\text{Na}^+$ . The points at both 1 and 5 mM  $\text{Na}^+$  are from 11 oocytes in wild type pumps and 15 oocytes in RD pumps.



**Supplementary Fig. 3. Effect of pH 4.0 on  $I_H$ .** **A**) Continuous current recording from an oocyte injected with ouabain-resistant mutant RD- $\alpha 1\beta 3$  pumps held at -50 mV. Following pump current activation by 3 mM K in external NMG solution the external pH was dropped to pH 4.0 inducing a biphasic response, similar to that at pH 5.0 (Fig. 7) but with more complete inhibition and with lower relative peak (probably due to the faster inhibition (rate limited by solution exchange) observed at the lower pH,  $4.9 \pm 0.4$  s,  $n=7$ ). Application of 125 mM Na activated an inward current that was inhibited by subsequent application of ouabain. **B**) Ouabain-sensitive current-voltage relationship for peak and steady state currents at pH 4.0 and 5.0, and for the steady state current in the presence of 5 mM Na and in 125 mM Na at pH 4.0. Currents were normalized to the K-induced current at -40 mV.