Supporting Information

Na/K pump mutations associated with primary hyperaldosteronism cause loss of function.

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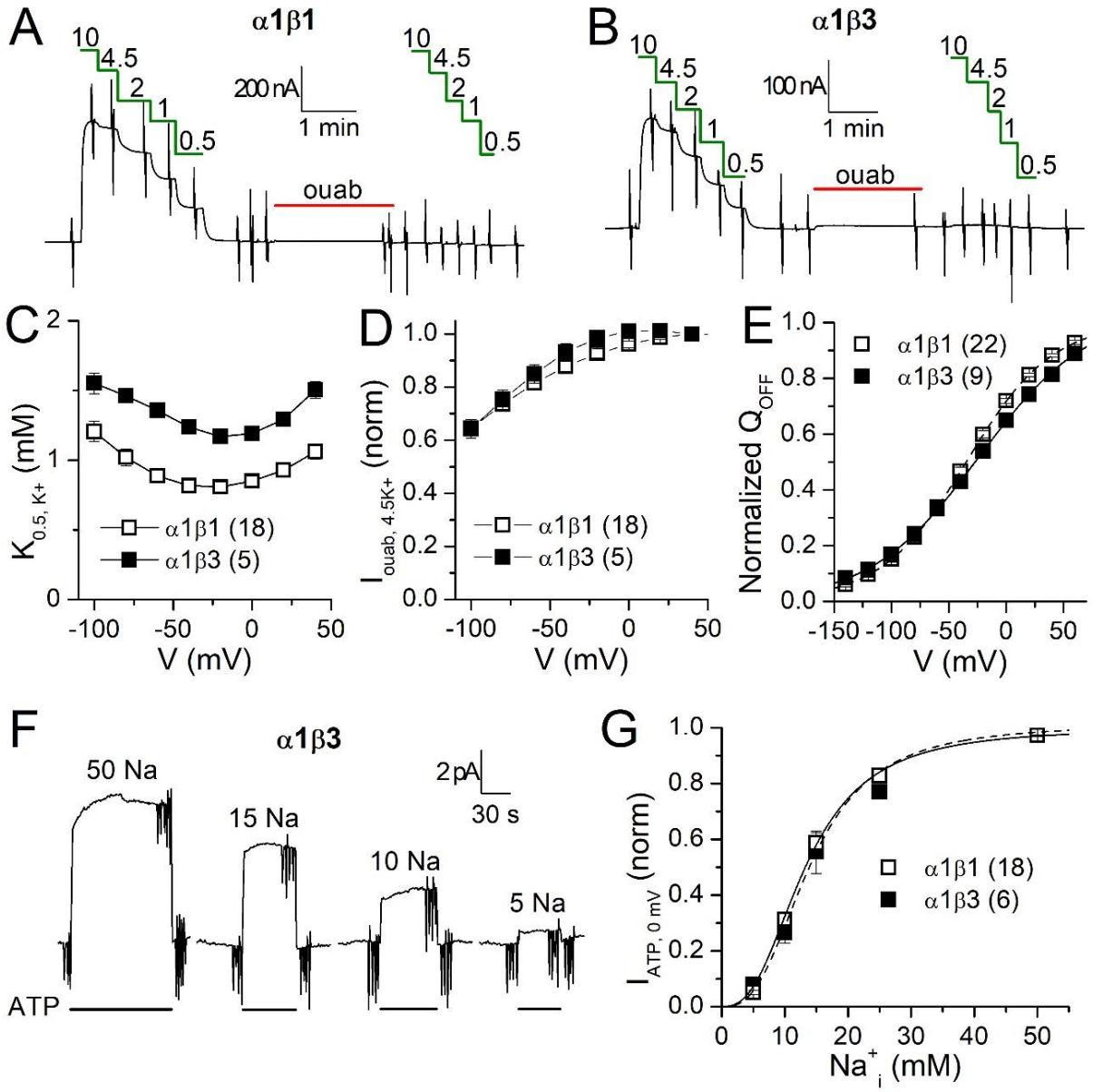

Figure S1. Comparison of wild type α1β1 and α1β3 pumps. **A and B)** Continuous TEVC recording of representative oocytes expressing α1β1 **(A)** or α1β3 **(B)**, held at -50 mV. Partial substitution of external Na^+ for K^+ activated outward current in a [K^+]-dependent manner$^+$ ([Na^+]+[K^+] = 150 mM, numbers indicate the millimolar [K^+]). Ouabain (ouab, 0.5 mM) inhibited current activation by subsequent K^+ applications. Vertical deflections correspond to 100 ms-long pulses to voltages between +40 and −140 mV, in 20 mV increments. **C)** Mean $K_{0.5,K+}$-V for α1β1 (open) and α1β3 (solid), obtained by fitting the external [K^+]-dependence of ouabain-sensitive current to a Hill equation (Methods). **D)** Mean ouabain-sensitive current in 4.5 mM K^+ for α1β1 (open) and α1β3 (solid), normalized to the outward current at +40 mV. **E)** Mean Q-V curves in 150 mM Na^+ for α1β1 (open) and α1β3 (solid, same as in Fig. 8), normalized to the maximum charge in each individual oocyte. Lines represent Boltzmann distributions (Methods) with

parameters $V_{1/2}$ = −34.4 ± 0.6 mV and kT/ez_q = 37.2 ± 0.7 mV for α1β1 (dashed), and $V_{1/2}$ = −23.6 ± 0.6 mV and kT/ez_q = 47.9 ± 1.2 mV for α1β3 (solid), obtained from fits to data from individual oocytes. **F)** ATP-activated currents recorded from an inside-out patch excised from an oocyte expressing α1β3 pump and held at zero voltage. Bars indicate application of 4 mM MgATP. The millimolar intracellular [Na^+] at the time of ATP application is indicated above each ATP-activated current. **G)** Mean intracellular Na^+-dependence of ATP-activated current from inside-out patches expressing α1β1 (open) or α1β3 (solid), normalized to the I_{max} (10.5 ± 1.0 pA for α1β1 and 4.6 ± 0.8 pA for α1β3) from the Hill fits. Data for α1β1 is the same as in Fig. 6. The symbols at 50 mM intracellular Na^+ are overlapping for both data sets. Line plots are Hill equations fitted to data for α1β1 (dashed) and α1β3 (solid), with best fit parameters (obtained from global fits to the raw data) $K_{0.5}$ = 14.0 ± 0.4 mM, *nH* = 2.75 ± 0.17 for α1β1 and $K_{0.5}$ = 13.2 ± 0.8 mM, *nH* = 2.62 ± 0.38 for α1β3). Parentheses indicate the number of averaged experiments in all panels.