

Supporting Information

Yaragatupalli et al. 10.1073/pnas.0903752106

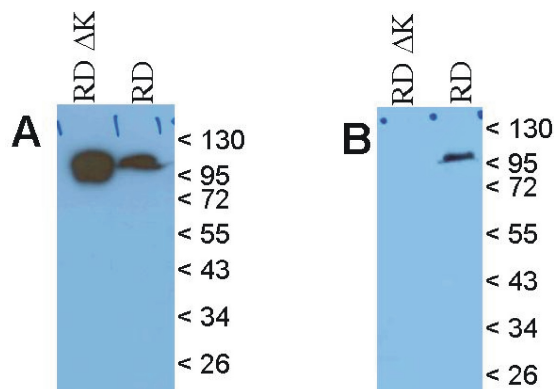


Fig. S1. Western blot analysis of Xenopus oocytes isolated plasma membrane proteins. (A) Immunodetection of both RD (right lane) and RD Δ KESYY (left lane, indicated RD Δ K) by an α 1 antibody. (B) Immunodetection with an antibody against the C terminus (anti-KETYY; gift from Jim Kyte, University of California, San Diego) which recognizes the Xenopus α 1 C-terminal sequence KESYY (right lane) and confirms the successful truncation of the mutant (left lane). Enriched plasma membrane fractions were obtained from \approx 40–50 oocytes according to the method of Hill et al. (1). Twenty-five mg of plasma membrane protein were separated via 10% SDS PAGE (2) and then electrotransferred to PVDF membrane in buffer containing 10 mM CAPS (pH = 11.0), 10% methanol, at constant current (180 mA) for 2 h (3). After blocking with 10% dry milk in PBS, the membrane was probed with anti-NKA antibody MA1–16731 (Affinity Bioreagents) (1:2,500), followed by incubation with HRP-conjugated goat anti-mouse 2^o antibody. Bands were detected via supersignal substrate kit (Pierce, Rockford, IL) according to the manufacturer's protocol.

1. Hill WG, et al. (2005) Isolation and characterization of the Xenopus oocyte plasma membrane: A new method for studying activity of water and solute transporters. *Am J Physiol Renal Physiol* 289:F217–F224.
2. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–685.
3. Matsudaira P (1987) Sequence from picomole quantities of proteins electroblotted onto polyvinylidene difluoride membranes. *J Biol Chem* 262:10035–10038, 1987.

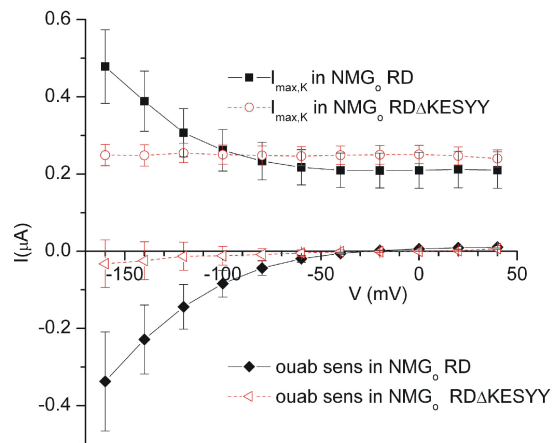


Fig. S2. Voltage dependence of Na/K pump related currents in NMG_o⁺. Maximal K_o⁺-induced current (I_{max}) from Hill fits (at zero Na_o⁺) are shown for RD (filled black squares, $n = 15$) and for RD Δ KESYY (open red circles, $n = 17$) pumps. Ouabain-sensitive currents (at zero K_o⁺) are plotted as a function of voltage for the RD pumps (filled black diamonds, $n = 9$) and for the RD Δ KESYY (open red triangles, $n = 10$). Note the presence of an inward current in RD pumps that disappears in RD Δ KESYY pumps.

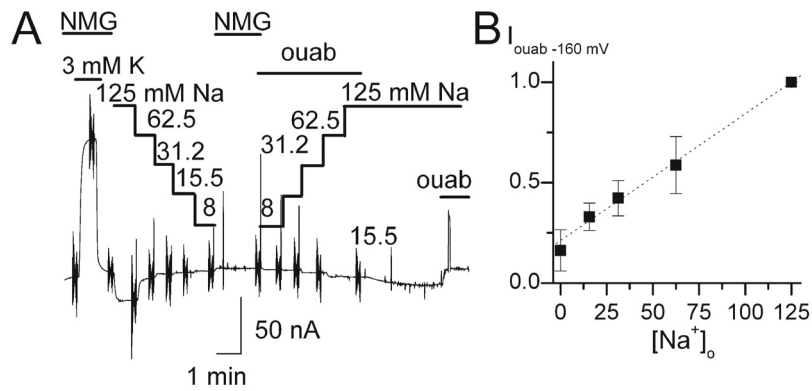


Fig. S3. $[\text{Na}^+]_o$ -dependence of inward steady state Na/K pump current at zero K^+_o . (A) Continuous TEVC recording illustrating the maneuvers performed to measure the $[\text{Na}^+]_o$ dependence of the inward leak through the Na/K pump at $V_h = -50$ mV. Sharp vertical deflections represent pulses from -200 mV to $+40$ mV applied to measure the current's voltage dependence. (B) $[\text{Na}^+]_o$ -dependence of the ouabain-sensitive steady state current measured at -160 mV normalized to the current observed in 125 mM Na^+_o . The line represents a linear fit to the average data from 5 oocytes.

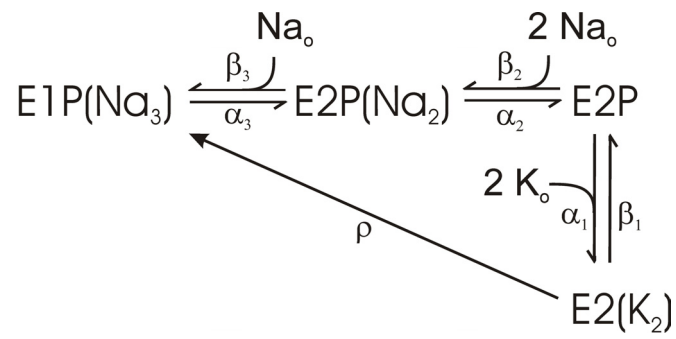


Fig. S4. Simplified kinetic scheme of Na/K pump function. Greek letters α and β indicate reaction rates in the forward and reverse directions, respectively, for each step in the scheme. For additional details, see [SI Appendix](#).

Other Supporting Information Files

[SI Appendix \(PDF\)](#)