SUPPLEMENTAL MATERIAL

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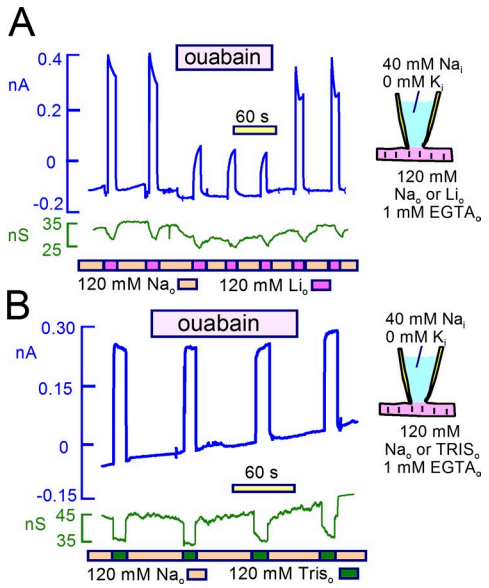

Figure S1. **Extracellular Na substitution cannot be used to define outward Na/Ca exchange current.** (A and B) Effects of substituting 120 mM extracellular Na for 120 mM Li (A) or 120 mM Tris (B) in the presence of 40 mM cytoplasmic Na in murine cardiac myocytes. (A) In the absence of Ca and K on both membrane sides, substitution of Na for Li activates an outward ouabain-sensitive current ~0.5 nA in magnitude. The current is inhibited ~70% by 200 µM ouabain, and it recovers for the most part upon wash off of ouabain. (B) Substitution of Na for Tris in an equivalent experiment results in an outward current shift that is *not* blocked by ouabain. This current shift probably reflects a decrease of Na current through the leak (seal) pathway, but it may also reflect the existence of bona fide nonselective cation channels in murine myocytes, as described in Fig. S3.

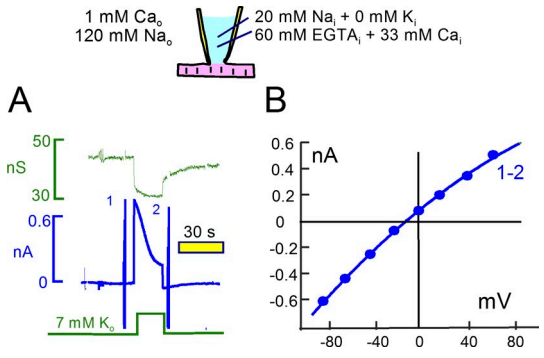

Figure S2. **Na/K pump activity modifies membrane conductance for prolonged times under conditions that enable reversible Na/Ca exchange function (i.e., with a highly Ca-buffered cytoplasmic solution, containing 60 mM EGTA with 33 mM Ca, and with 20 mM cytoplasmic Na and 120 mM extracellular Na and 1 mM extracellular Ca).** (A) The activation of Na/K pumps for 30 s causes a substantial reduction of cell conductance that reverses in a rapid and a slow phase after termination of pump current. (B) The current that is apparently blocked by pump activity reverses at −20 mV. This outcome cannot be explained by Na/K pumps causing a depletion of cytoplasmic Na, because in that case, the subtracted current would not reverse. The result is more consistent with Na/K pumps causing a genuine decrease of Na/Ca exchange activity, but this cannot be proved with available pharmacological tools.

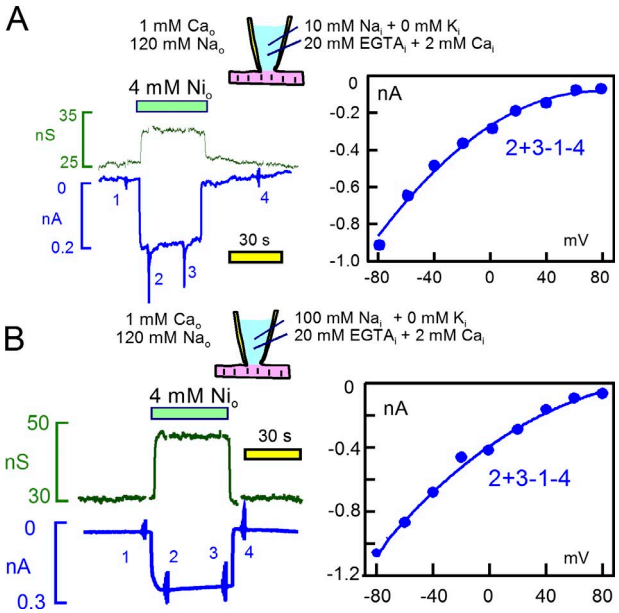

Figure S3. **Ni cannot be used to define Na/Ca exchange current-voltage relations in murine myocytes.** (A) Ni activates a significant inward current and conductance in murine myocytes. Current-voltage relations (right) reveal that the current activated by Ni does not reverse in the potential range of −80 to +80 mV in murine myocytes. (B) The inward current activated by Ni is similar when 100 mM Na is used in pipette solutions. It does not reverse up to +80 mV. Thus, the Ni current is likely a genuine divalent cation current that is unrelated to Na/Ca exchange function. Presumably, this current reflects a nonselective cation conductance that may explain why Na concentrations in myocytes can be substantially lower than expected from pipette Na concentrations (see Fig. S4) when Na gradients are present.

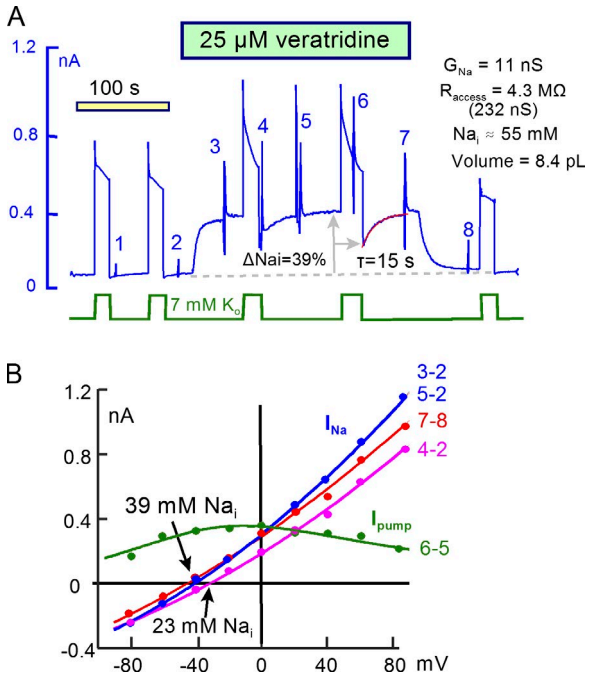

Figure S4. **Na/K pump activity causes significant Na depletion when high cytoplasmic Na concentrations (60 mM) are used and Na/K pump inactivation is attenuated.** (A) Current record using the same conditions and protocol as in Fig. 9 B (7 mM extracellular Na), but including aquisition of current–voltage relations to determine subsarcolemmal Na changes. (B) Current–voltage relations without veratridine (2 and 8) were used as baseline to determine current–voltage relations for the veratridine-dependent Na currents (3, 4, 5, and 7). In addition, the current voltage relation of the Na/K pump current was determined (6–5) and shows, as expected for the experimental conditions, almost only weak voltage dependence. The current–voltage relation illustrated was generated by the simulation program used to predict data shown in Fig. 5. The initial reversal potential of Na current in veratridine indicates a subsarcolemmal Na concentration of 39 mM, versus the pipette concentration of 60 mM. The apparent decrease of Na as a result of channel activity is twice larger than expected for the 320 pA outward current activated by veratridine. Pump activity shifts the Na current reversal potentials from −45 mV to −34 mV, corresponding to a decrease of Na from 39 to 23 mM. Given that the pump current amounts to more than 0.5 nA (i.e., 1.5 nA equivalent Na flux), this decrease is closely consistent with the simple model. The τ of 15 s projects to a cytoplasmic volume of 8.4. pL.