Supporting Information

John et al. 10.1073/pnas.1218751110

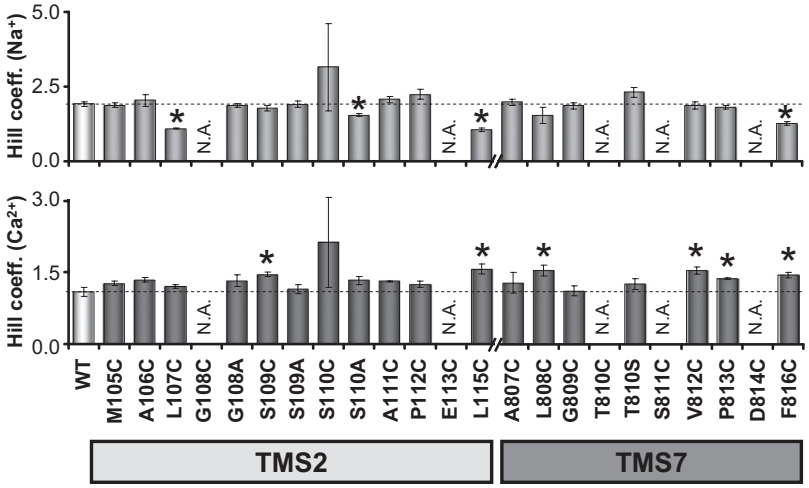

Fig. S1. Properties of mutated Na^+-Ca^{2+} exchangers (NCXs). Hill coefficient values for NCX1.1 mutants investigated in this study. *Upper* shows values (given as mean ± SE) obtained by fitting the Na^+ dependency curves, whereas *Lower* shows values related to Ca^{2+} binding. Values statistically different from WT are marked with an asterisk ($P < 0.05$). Mutants with no measurable activity are indicated as N.A.

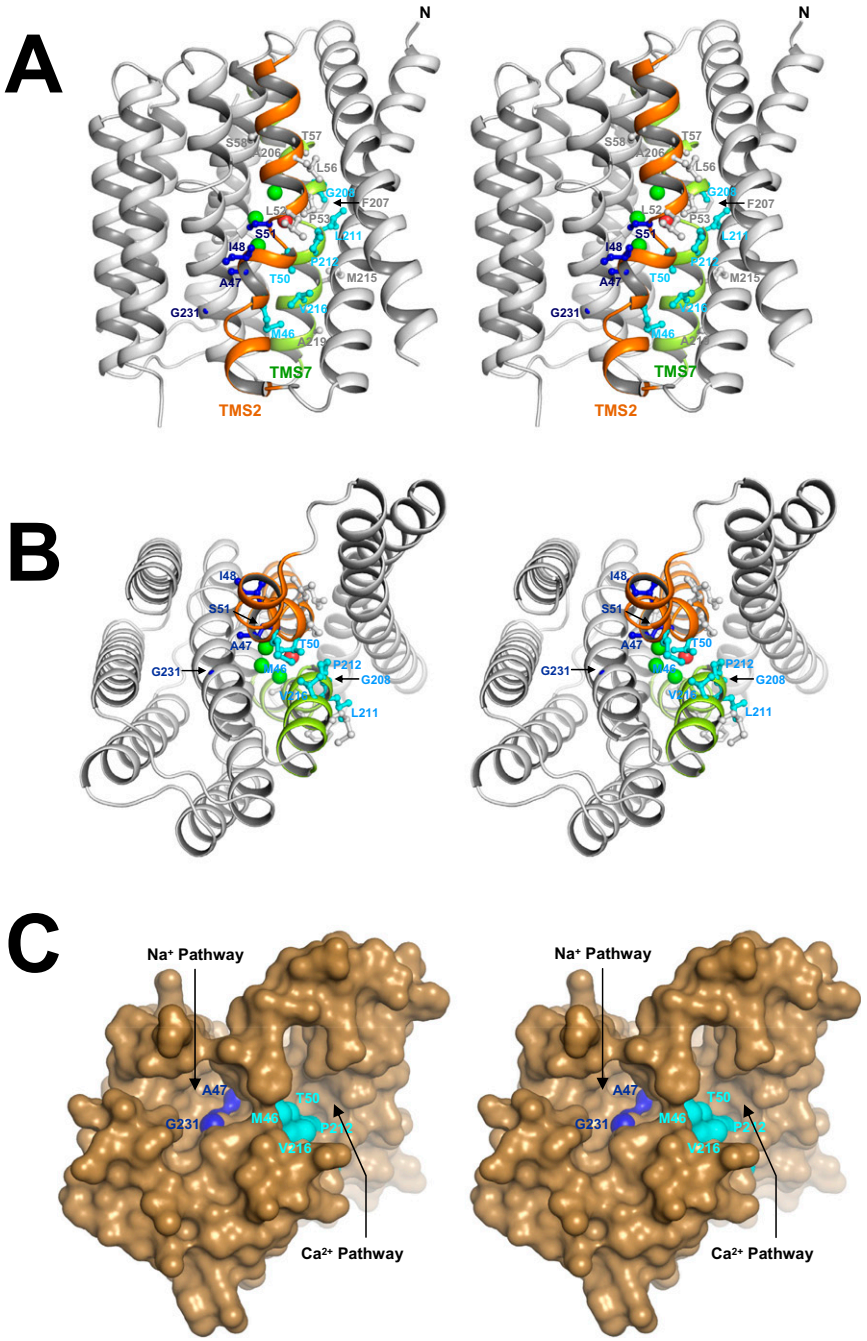

Fig. S2. Inward-facing model of archaebacterial NCX homolog (NCX_Mj) showing surface representation and residue accessibility. Stereoview of the inward-facing NCX_Mj model from the membrane (*A*) and the cytoplasm (*B*). Residues found to react with 2-(Trimethylammonium)ethyl Methanethiosulfonate Bromide ($MTSET^+$) when the ion binding sites face the cytoplasm (inward configuration) are in blue. Residues accessible to $MTSET^+$ both during transport and in the inward-facing state in cyan. Unreactive residues are shown in gray. More details can be found in Fig. 6. A surface representation of NCX_Mj (inward-facing configuration) as seen from the cytoplasmic side is shown in *C*. The inward state was modeled as described previously (1). Residues accessible from the cytoplasm are colored as described above. Note the presence of two independent cavities accessible from the cytoplasmic side.

1. Liao J, et al. (2012) Structural insight into the ion-exchange mechanism of the sodium/calcium exchanger. *Science* 335(6069):686–690.

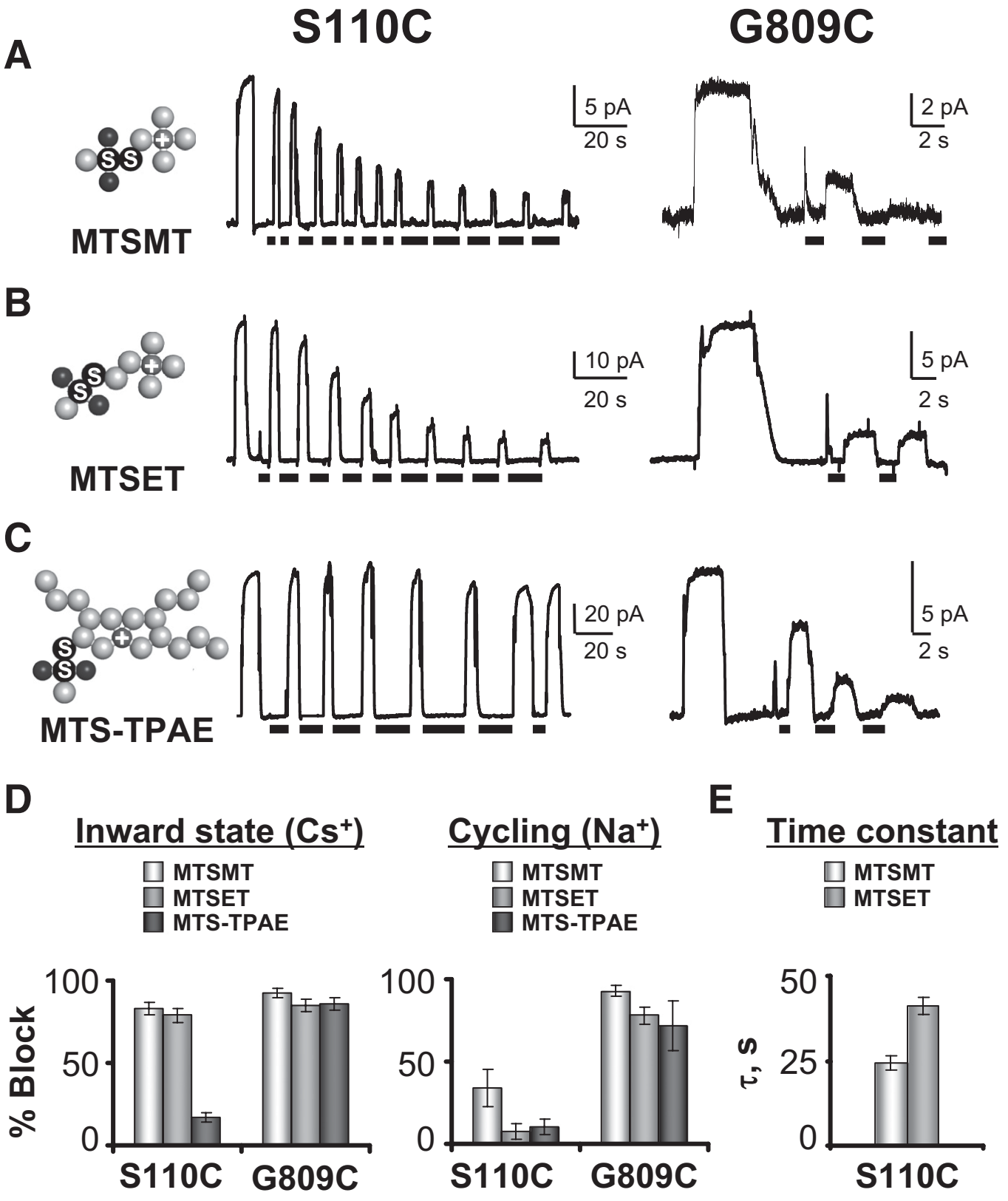

Fig. S3. The two cytoplasmic cavities of NCX1.1 have different sizes. Molecular models for 1-(Trimethylammonium)methyl Methanethiosulfonate Bromide ($MTSMT^+$; *A*), $MTSET^+$ (*B*), and 2-(Tripentylammonium)ethyl Methanethiosulfonate Bromide (MTS-TPAE; *C*) reagents. $MTSMT^+$ is slightly shorter than $MTSET^+$ because of the presence of a methyl group instead of an ethyl chain. In contrast, the membrane-impermeable MTS-TPAE is significantly larger, being almost two times the size of both $MTSMT^+$ and $MTSET^+$. The effects of $MTSMT^+$ (5 mM; *A*), $MTSET^+$ (5 mM; *B*), and MTS-TPAE (100 μM; *C*) on NCX1.1 currents when held in the inward configuration state are depicted. $MTSMT^+$ inhibited S110C exchanger current more rapidly than $MTSET^+$, supporting the hypothesis of

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restricted accessibility at this location. The large MTS-TPAE did not cause S110C current inhibition while effectively blocking G809C currents. These results indicate that cysteines 110 and 809 are exposed to crevices of different sizes. The extent by which MTS regents inhibited NCX1.1 mutant currents is summarized in *D*, whereas the rate of S110C block by $MTSMT^{+}$ and $MTSET^{+}$ is shown in *E*.