John et al., https://doi.org/10.1085/jgp.201611693

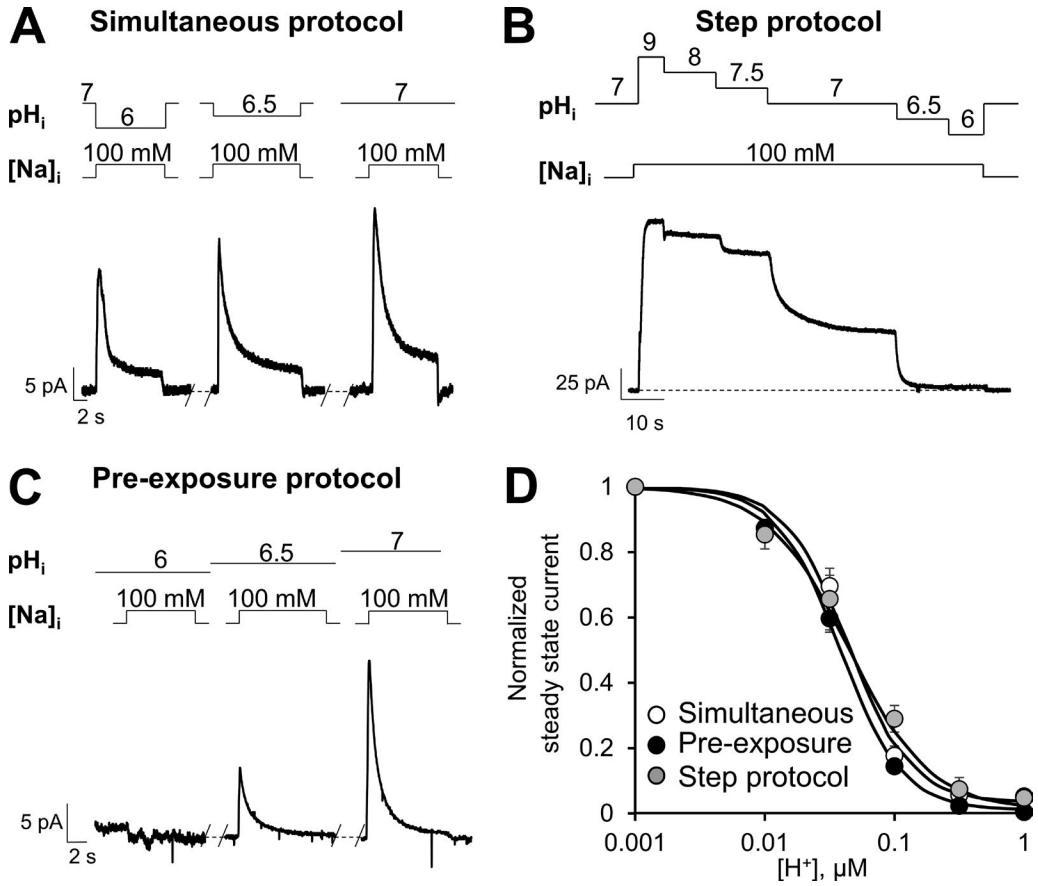

Figure S1. **Protocols used to determine NCX pH sensitivity.** Outward exchange currents from WT were recorded using the indicated protocols. Protocols are further described within the methods section. The pH sensitivity of the large library of mutants was first investigated by assessing the apparent proton affinity of NCX at steady-state. **(A and B)** For this purpose, the protocols in A (simultaneous) and B (step) were used interchangeably and often within the same patch. These protocols are advantageous because they minimize the exposure of the patch to stressful pH, favoring a higher success rate. **(C)** Mutants of interest were further investigated with a protocol entailing preexposure of the patch to various pHs (20–25 s) before the Na^+ pulse (preexposure protocol). This protocol allows determination of the effects of pH both at the onset of the current (peak current, before Na^+-dependent inactivation has developed) and at steady state (after the Na^+-dependent inactivation has occurred). Please note that in mutants lacking Na^+-dependent inactivation (any exchanger carrying mutation at site 165 or 229), the steady-state and peak currents are the same. We limited this protocol to the mutants of interest, because it is technically challenging owing to the long exposures of stressful pH, which limits the success rate of completed protocols. **(D)** Independent of the protocol used, the sensitivity of NCX to cytoplasmic protons, once it reaches steady state, is the same. Error bars represent SEM.

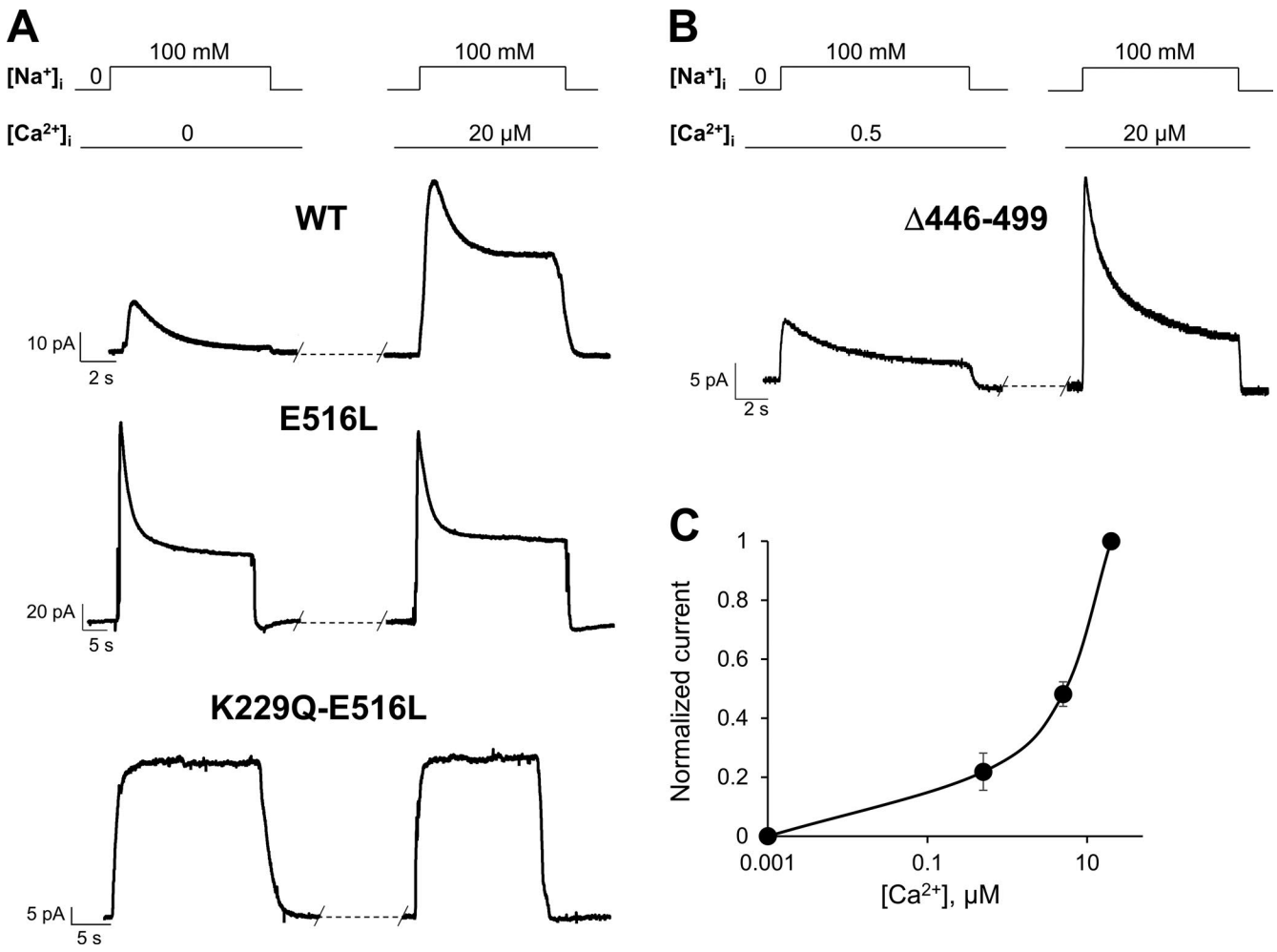

Figure S2. **Mutation of Lys 229 and Glu 516 removes both Na^+ and Ca^+ regulation. (A)** Examples of giant-patch recordings from oocytes expressing the indicated exchangers. Outward currents were generated by rapidly applying 100 mM Na^+ to the bath (intracellular surface) with 8 mM Ca^{2+} in the pipette while maintaining the pH constant at 7. Representative traces in the presence of two different intracellular Ca^{2+} concentrations are shown. The indicated free calcium concentrations were obtained by adding Ca^{2+} buffers (HEDTA or EGTA) to the bath solution. Although WT exchange currents peaked and then slowly decayed because of Na^+-dependent inactivation, the K229Q-E516L mutant failed to inactivate in the presence of high intracellular Na^+. Furthermore, micromolar cytoplasmic Ca^{2+} concentrations eliminated Na^+-dependent inactivation in WT and potentiated the current. In contrast, application of Ca^{2+} had no further effect on the double mutant. Thus, K229Q-E516L was free of both Na^+- and Ca^{2+}-dependent allosteric effects. Similarly to K229Q-E516L, high intracellular Ca^{2+} did not affect E516L peak or steady-state currents, as previously demonstrated (Besserer et al., 2007). **(B and C)** Deletion of residues 446 to 499 within CBD1 drastically decreased the sensitivity of NCX to cytoplasmic Ca^{2+}. The corresponding dose–response curve for cytoplasmic Ca^{2+} is shown in C. Current amplitudes were measured at peak. The Δ446–499 peak current was normalized at the highest concentration of Ca^{2+} examined, because saturation was not obtained. Each point is the mean of two experiments, and the SD is shown.

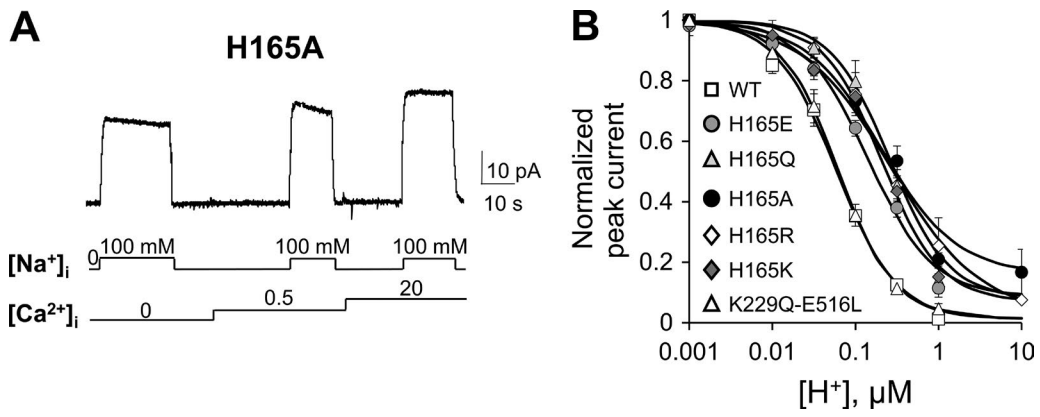

Figure S3. **His 165 is part of NCX pH sensor. (A)** Mutation of His 165 removes Na^+ and Ca^{2+} regulation and drastically affects NCX pH sensitivity. Representative traces were recorded at the indicated cytoplasmic Ca^{2+} concentrations. The desired final free concentration of Ca^{2+} in the bath was achieved by adding calcium buffers (10 mM EGTA or HEDTA) to solutions with different $Ca(OH)_2$ concentrations. The data indicate that H165 is a strategic residue in NCX allosteric pH regulation. **(B)** Dose–response curves for cytoplasmic H^+ for WT and mutant exchangers K229Q-E516L, H165R, H165K, H165Q, H165A, and H165E were measured at peak. Note that mutations at position 165 all decreased NCX sensitivity to intracellular pH independently of properties of the side chain. Error bars represent SEM.

REFERENCE

Besserer, G.M., M. Ottolia, D.A. Nicoll, V. Chaptal, D. Cascio, K.D. Philipson, and J. Abramson. 2007. The second Ca^{2+}-binding domain of the Na^+ Ca^{2+} exchanger is essential for regulation: crystal structures and mutational analysis. *Proc. Natl. Acad. Sci. USA*. 104:18467–18472. https://doi.org/10.1073/pnas.0707417104