

Supplementary Text

Inhibition of S532C by MTSET at intracellular pH 6.8 indicates accessibility in the closed state

It is difficult to examine accessibility of cysteine-substituted mutants in the fully closed state using whole-cell recordings. We therefore examined accessibility of S532C in the fully closed state using inside-out recordings (Supplementary Figure 5). Membrane seals for cell-attached configuration were established quickly (~ 30 s) with MTSET in the patch pipettes. Immediately after, inside-out membranes were excised into pH 9-cytoplasmic solutions to avoid significant modification by MTSET at the resting pH_i. After currents stabilized at pH 9.0, inside-out membranes were exposed to pH 4 to fully close the channels. Thereafter, membranes were exposed to pH 9.0 to see channels can be reopened after exposing to extracellular MTSET during the closed state. For wild type TRPV5, currents were completely inhibited at pH 4 but reversed at pH 9.0 (Supplementary Figure 5A). In contrast, currents from the S532C channel did not reverse at pH 9.0 after inhibition by pH 4.0 (Supplementary Figure 5B). These results confirm that amino acid S532 is not accessible in the open state but becomes accessible to MTSET in the closed state. In similar inside-out recording experiments, we also confirmed that E535C is not accessible in the open state but is accessible in the closed state (not shown).

Supplementary Figures

Supplementary Figure 1. Effects of intracellular pH on TRPV5 single channel properties. (A) Representative single channel recording of TRPV5 at pH_i 7.0 or 8.4. Only one active channel was

present in the excised patch during 20 minute continuous recording. Holding potential at -50 mV.

(B) Effect of intracellular pH on single channel open probability of TRPV5. Excised inside-out patches were exposed sequentially to pH_i 8.4, 7.0, and back to 8.4. Recordings were excluded for analysis if significant run down of channel activity occurred ($>10\%$ difference between the first and last pH_i 8.4). $N\text{Po}$ is number of channels (N) x single channel open probability (P_o). Mean \pm SEM, $n = 6$. **(C)** Effect of intracellular pH on single channel conductance. Slope conductance was determined from single channel current amplitude (pA) vs holding potentials (from -25 to -75 mV). For excised inside-out single-channel recording (Yeh et al., 2003), pipette and bath solution contain 140 NaAsp, 10 NaCl, 1 EDTA, HEPES 10 (pH 7.4) and 140 NaAsp, 10 NaCl, 1 EDTA, 10 HEPES (pH 7.0 or 8.4), respectively. Currents were low-pass filtered at 1 kHz using an 8-pole Bessel filter, sampled every 0.1 ms (10 kHz) with Digidata-1300 interface and stored directly onto computer hard disk using pCLAMP9 software. Data were transferred to CD for long-term storage. Single channel current amplitude, histogram, and open probability were analyzed using Clampfit9 program of pCLAMP9 software (Axon Instruments) (Yeh et al., 2003).

Supplementary Figure 2. Regulation of TRPV5 by intracellular pH at extracellular pH 8.4 or 6.8.

(A) Current-voltage relationships of currents (inside-out recording at pHe 8.4) at different intracellular pH (pH_i). **(B)** Inward currents (pA at -100 mV; inside-out recording at pHe 8.4) at different pH_i over time. **(C)** Current-voltage relationships of currents (inside-out recording at pHe 6.8) at different pH_i . **(D)** Inward currents (pA at -100 mV; inside-out recording at pHe 6.8) at different pH_i over time.

Supplementary Figure 3. Relationships of the relative permeability ratios of permeating ions over

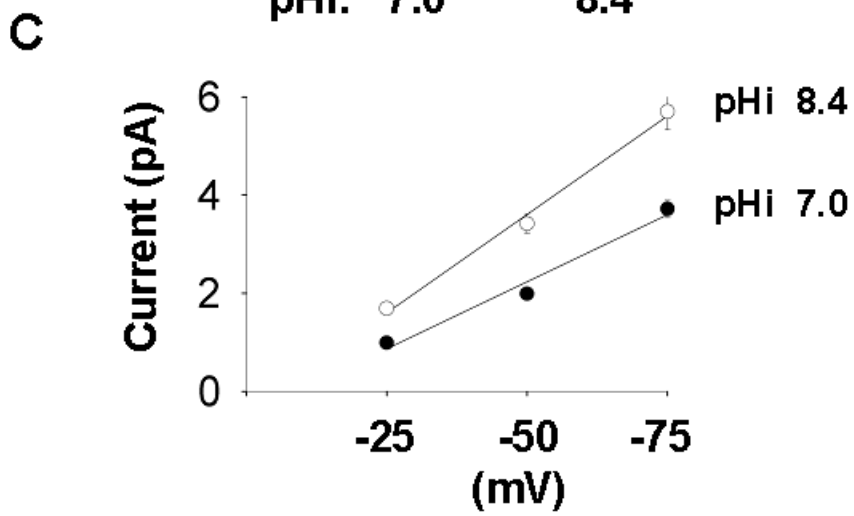
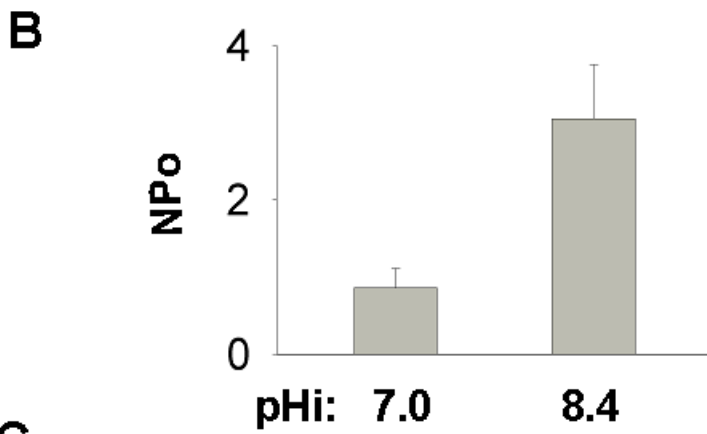
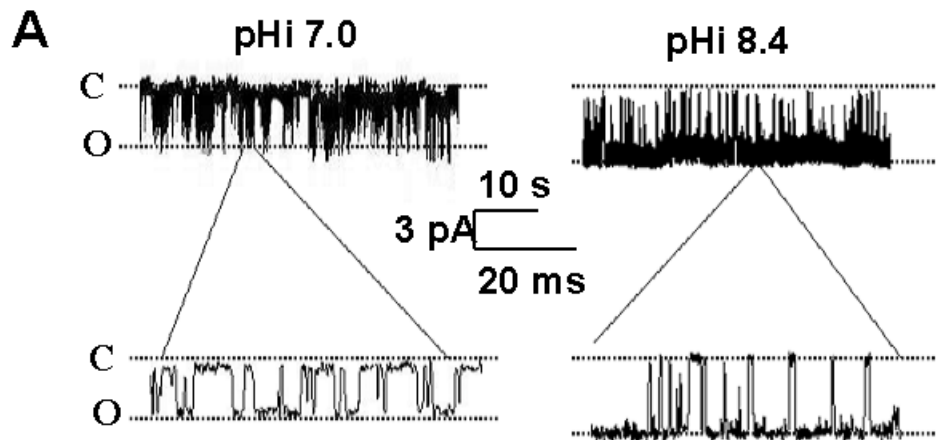
Na⁺ vs diameter of permeating ions at different intracellular pH's for wild type TRPV5 (**A**), at different extracellular pH's for wild type TRPV5 (**B**), and at different intracellular pH's for D542A TRPV5 mutant (**C**). The relative permeability ratios (P_X/P_{Na}) were calculated according to the equation $P_X/P_{Na} = \exp(\Delta E_{rev} \times F/RT)$. ΔE_{rev} is the shift of reversal potential upon changing bath solution from NaAsp to X-Asp. X is MA, DMA or NMDG with a diameter of 3.6, 4.6, and 6.8 Angstrom, respectively. Data points were fitted according to the excluded volume equation $P_X/P_{Na} = k(1-a/d)^2/a$ (Dwyer et al., 1980; Voets et al., 2004), where a is the diameter of MA⁺, DMA⁺ or NMDG⁺, and d the minimal pore diameter.

Supplementary Figure 4. Shift of reversal potential (ΔE_{rev}) upon changing from Na⁺- to Ca²⁺-containing bath solution at intracellular pH 9.0 (**A**), 7.4 (**B**) or 6.0 (**C**). Whole-cell TRPV5 currents (I-V curves) were first recorded at 140 mM NaAsp bath solution (labeled as Na⁺) and subsequently changed to 130 mM NaAsp + 10 mM CaCl₂ (labeled as Ca²⁺). Upward arrow indicates reversal potentials in 130 NaAsp + 10 CaCl₂-containing bath solution.

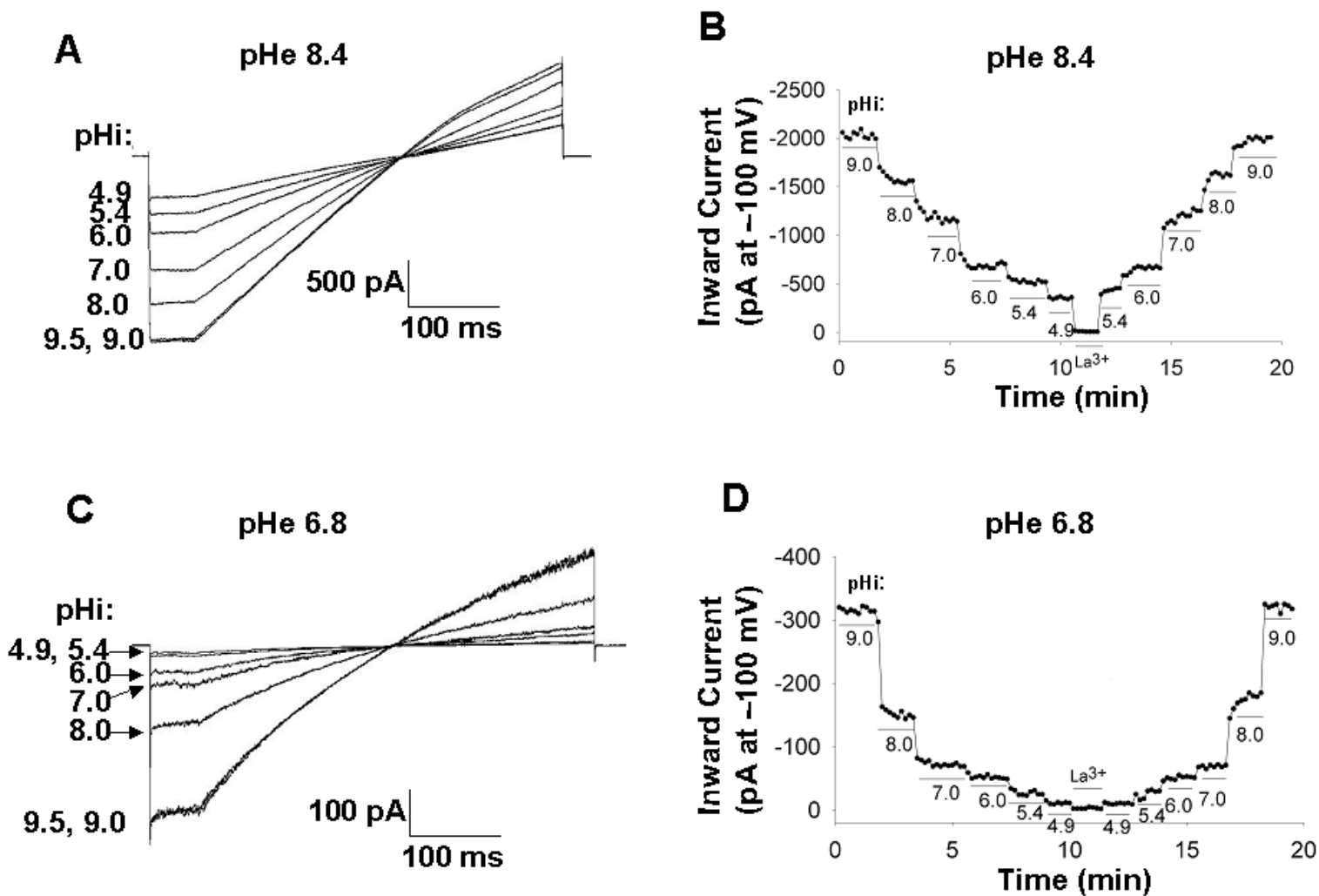
Supplementary Figure 5. Amino acid S532 is accessible in the fully closed state. Accessibility to MTSET (included in the patch pipette) in the fully closed state for wild type (**A**) and S532C mutant (**B**) was examined in inside-out recordings by exposing to pH_i 4.0. Shown are inward currents (at -100 mV) from voltage ramps applied every 10s.

Reference

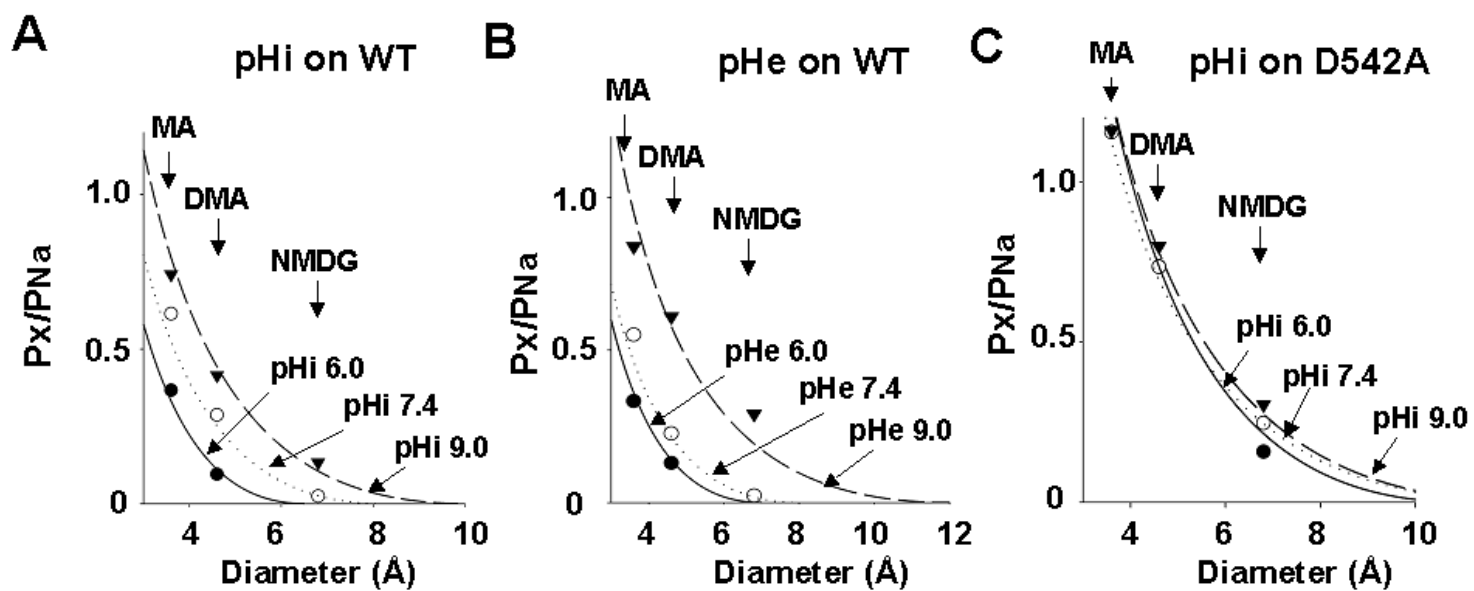
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- Voets, T., Janssens, A., Droogmans, G., and Nilius, B. (2004) Outer pore architecture of a Ca²⁺-selective TRP channel. *J. Biol. Chem.* **279**, 15223-15230.
- Yeh, B.-I., Sun, T.-J., Lee, J. Z., Chen, H.-H., and Huang, C.-L. (2003) Mechanism and molecular determinant for regulation of rabbit transient receptor potential type 5 (TRPV5) channel by extracellular pH. *J. Biol. Chem.* **278**, 51044-21052.



Supplementary Figure 1

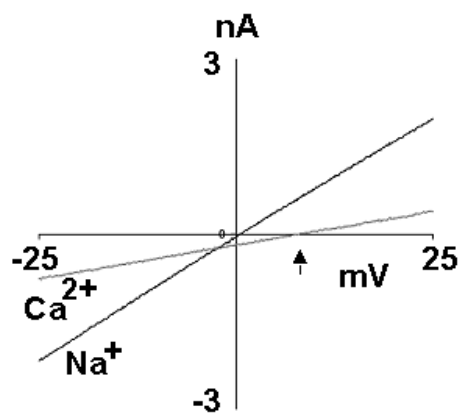


Supplementary Figure 2

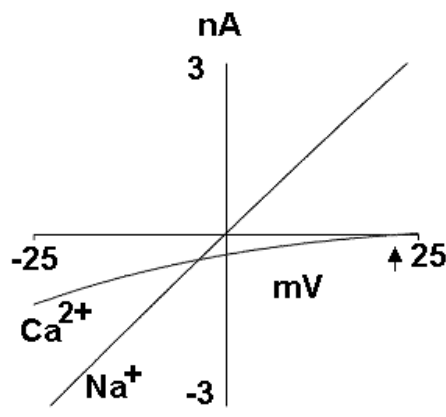


Supplementary Figure 3

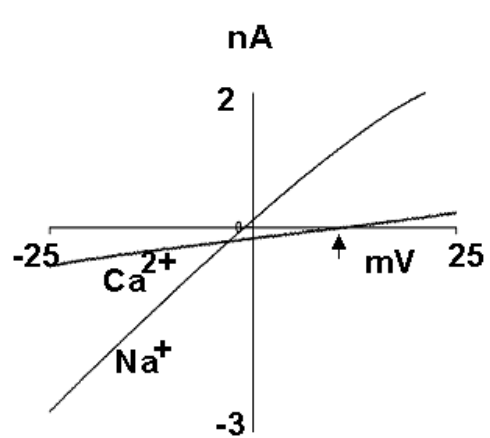
A pHi 9.0



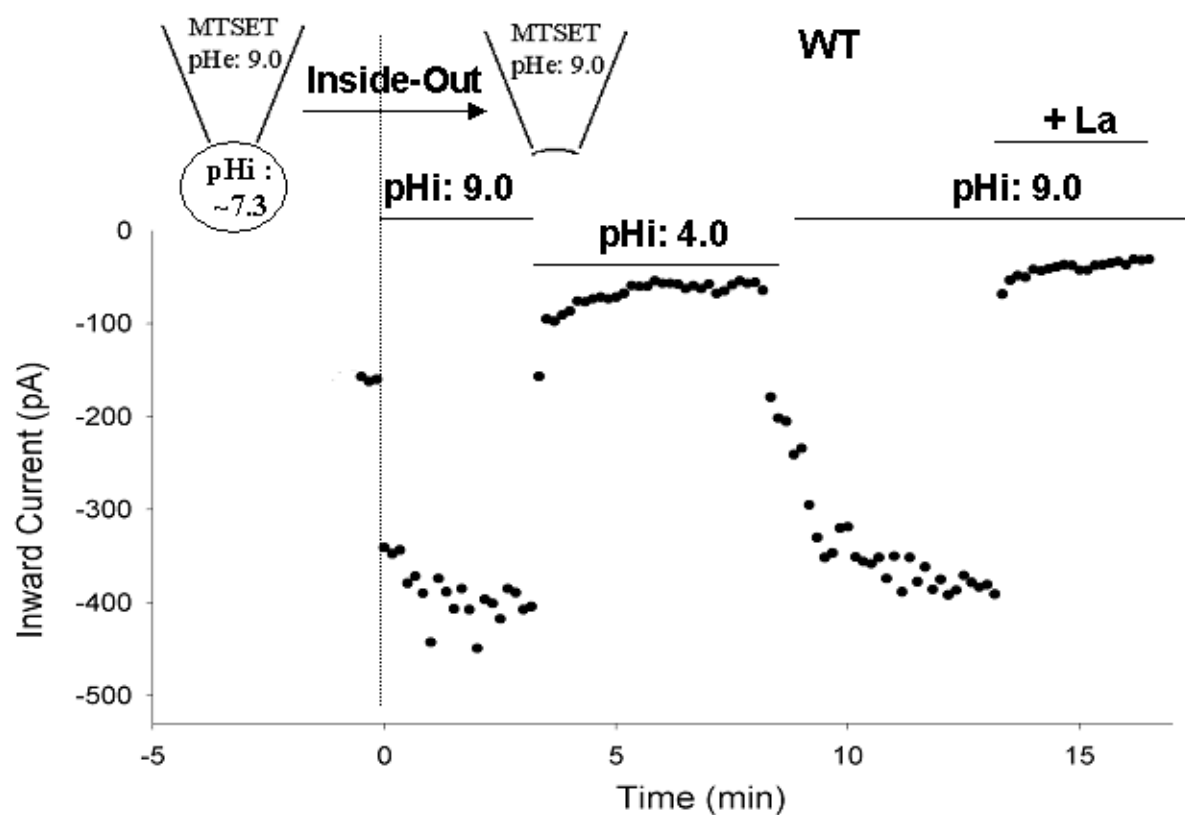
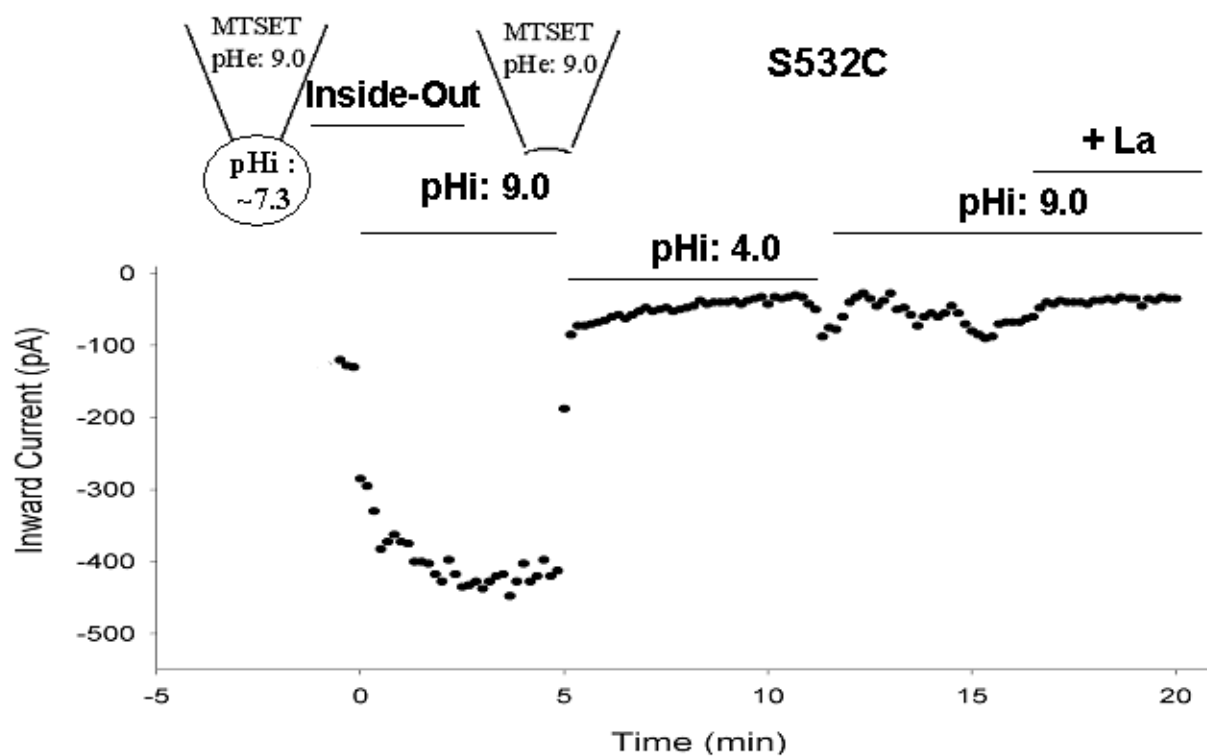
B pHi 7.4



C pHi 6.0



Supplementary Figure 4

A**B****Supplementary Figure 5**