iScience, Volume *24*

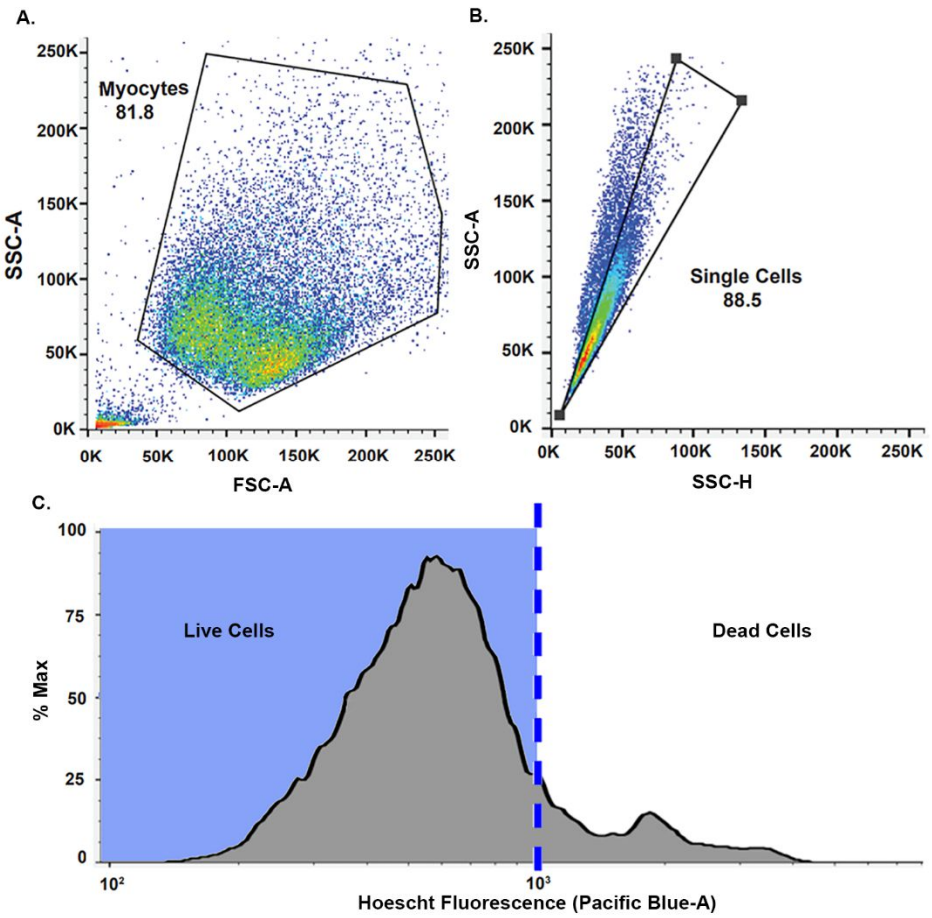
Supplemental information

SLO2.1/NALCN a sodium signaling complex that regulates uterine activity

Juan J. Ferreira, Chinwendu Amazu, Lis C. Puga-Molina, Xiaofeng Ma, Sarah K. England, and Celia M. Santi

Supplementary Figures.

Figure Supplementary 1: Optimized flow cytometry parameters. Related to STAR Methods. (A) SSC-A and FSC-A were used to identify MSMCs primarily by size. **(B)** SSC-A and SSC-H were used to select singlets and exclude doublets or debris. **(C)** Hoechst dye was used to differentiate between live and dead MSMCs.



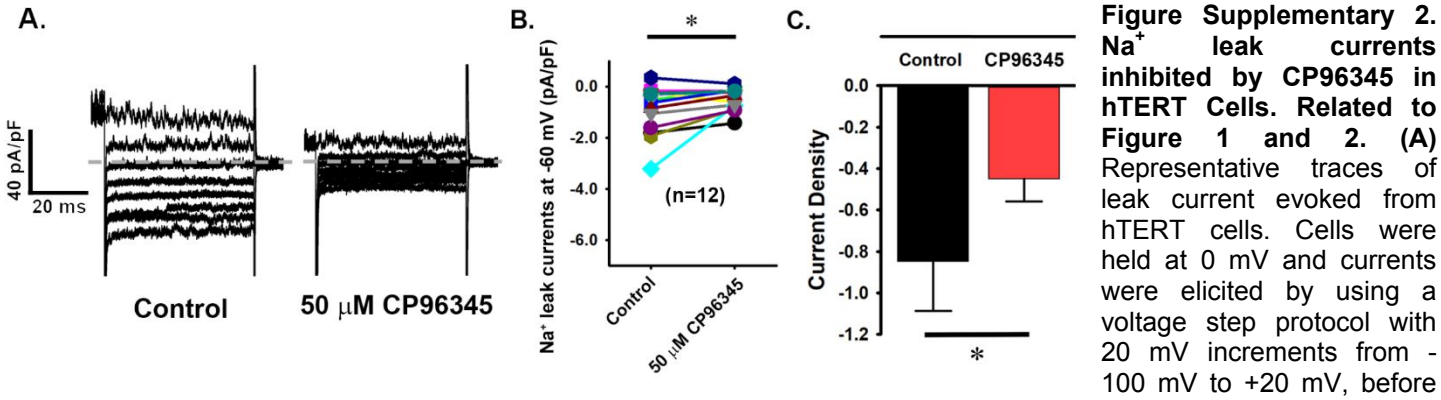


Figure Supplementary 2. Na^+ leak currents inhibited by CP96345 in hTERT Cells. Related to Figure 1 and 2. (A) Representative traces of leak current evoked from hTERT cells. Cells were held at 0 mV and currents were elicited by using a voltage step protocol with 20 mV increments from -100 mV to +20 mV, before and after treatment with 50 μM CP96345 (CP). Capacitive currents were removed from these traces. **(B)** Na^+ leak currents at -60 mV obtained before (control) and after treatment with 50 μM CP. Symbols represent individual cells and connecting lines link values in the same cell sample before and after treatment (n=12). **(C)** Current density analysis before and after treatment with 50 μM CP (n=12). Data are presented as mean values and SD. Cells were serum-starved in 0.5% FBS-containing media for 20–24 hours before experiments were performed. Pipettes were filled with a solution containing 125 mM Cs-Aspartate, 20 mM tetraethylammonium (TEA)-Cl, 5 mM Mg-ATP, 5 mM EGTA, 100 nM free Ca^{2+}, and 10 mM HEPES, pH 7.2. Currents were measured in an extracellular solution containing: 125 mM NaCl, 20 mM TEA-Cl, 0.1 mM $MgCl_2$, 5 mM HEPES, 11 mM glucose, 1 mM $CaCl_2$, and 5 mM nifedipine, pH 7.4.* P<0.050 by paired t-test.

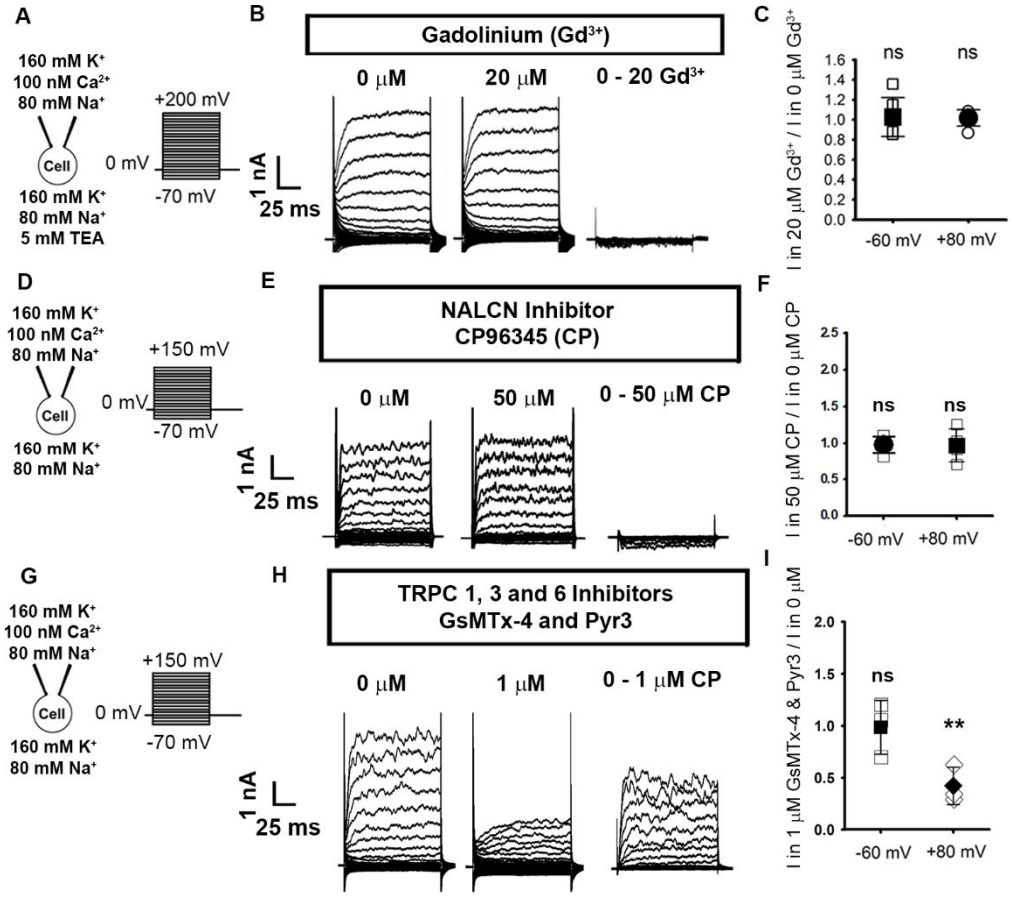
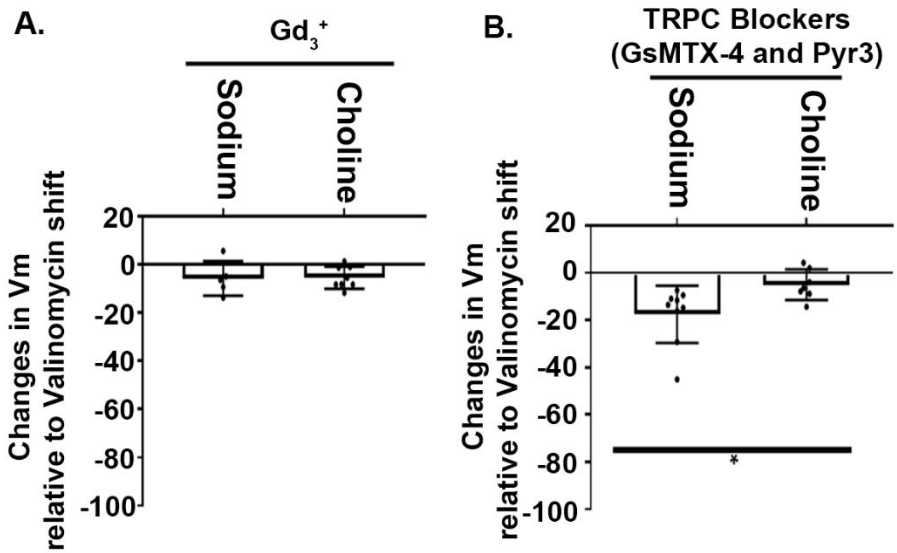


Figure Supplementary 3. Effects of Gadolinium, CP96345 and GsMTx-4 and Pyr3 over SLO2.1 currents. Related to Figure 1 and 2. (A) Schematic of whole cell recording set-up and voltage steps applied. **(B)** Representative whole-cell currents (V_h = 0 mV, with step pulses from −80 to +150 mV) from human MSMC recorded in 0 or 20 μM Gd^{3+}. **(C)** Graph of currents in the presence of 20 μM Gd^{3+} at -60 mV and +80 mV, normalized to currents in 0 μM Gd^{3+} and presented as mean and standard deviation. Values are 1.03 ± 0.195 at -60 mV (n=6, *P* = 0.739) and 1.018 ± 0.0814 at +80 mV (n= 6, *P* = 0.589). **(D)** Schematic of whole cell recording set-up and voltage steps applied. **(E)** Representative whole-cell currents (V_h = 0 mV, with step pulses from −80 to +150 mV) from human MSMC recorded in 0 or 50 μM CP. **(F)** Graph of currents in the presence of 50 μM CPat -60 mV and +80 mV, normalized to currents in 0 μM CP and presented as mean and standard deviation. Values are 0.976 ± 0.119 at -60mV (n=6) and 0.972 ± 0.237 at +80 mV (n= 6). *P*-values calculated by paired t-test. ns, not significant**. (G)** Schematic of whole cell recording set-up and voltage steps applied. (H) Representative whole-cell currents (V_h = 0 mV, with step pulses from −80 to +150 mV) from human MSMC recorded in 0 or 1 μM GsMTx-4 and Pyr3. (I) Graph of currents in the presence of 1 μM GsMTx-4 and Pyr3at -60 mV and +80 mV, normalized to currents in 0 μM and presented as mean and standard deviation. Values are 0.983 ± 0.259 at -60mV (n=3) and 0.419 ± 0.179 at +80 mV (n= 3). *P*-values calculated by independent t-test. ** *P*<0.010; ns = not significant.

Figure Supplementary 4: Gadolinium blocks hyperpolarization caused by extracellular Na^+, whereas TRPC blockers only partially decrease the hyperpolarization caused by extracellular Na^+. Related to Figure 2. (A) Quantification of shifts in DiSC3(5) fluorescence caused by Sodium (n= 5) and Choline (n=6) in the presence of Gd^{3+}. **(B)** Quantification of shifts in DiSC3(5) fluorescence caused by Sodium (n=9) and Choline (n=7) in the presence of GsMTx-4 and Pyr3. In **(A)** and **(B)**, values are normalized to shifts in fluorescence in the presence of valinomycin. Data are presented as mean and standard deviation. **P*<0.05 by unpaired t-test with Mann-Whitney corrections.



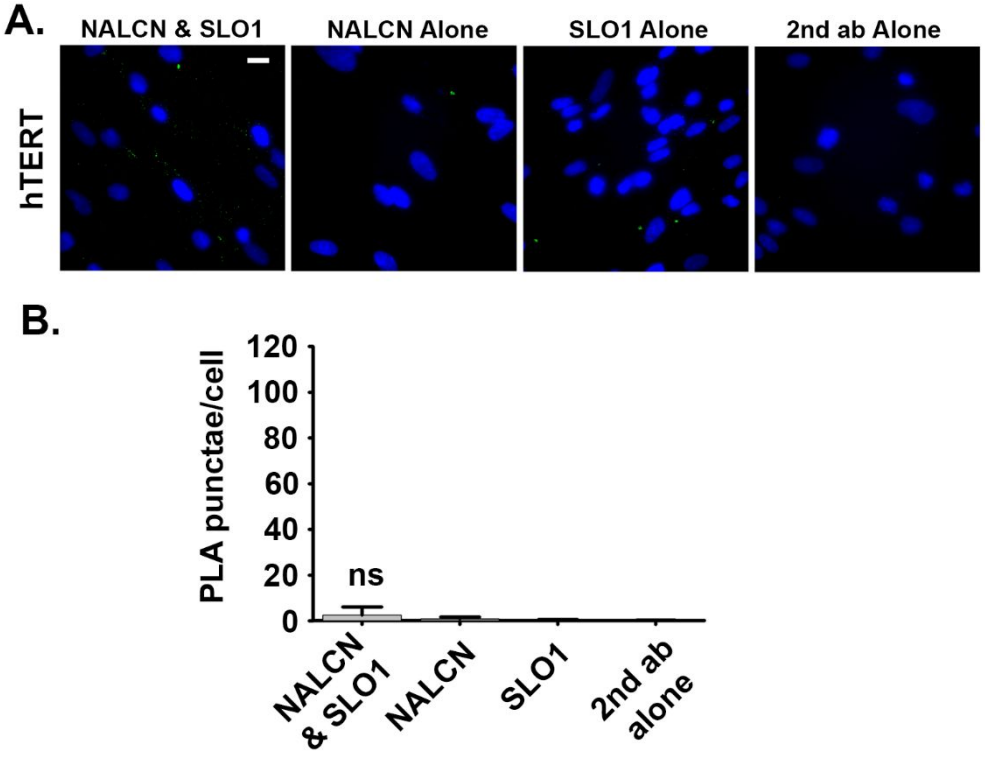


Figure Supplementary 5: NALCN and SLO1 are NOT in proximity in human MSMCs cells. Related to Figure 4.(A) Representative proximity ligation assay (PLA) labeling of hTERT-HM with the indicated single antibodies and antibody combinations. (Scale bar, 20 μm.) **(B)** Average number of PLA punctae in hTERT-HM cells (n=2-3). Over 200 cells per condition were processed. Data are presented as mean and standard deviation. ns>0.05 by One way ANOVA.

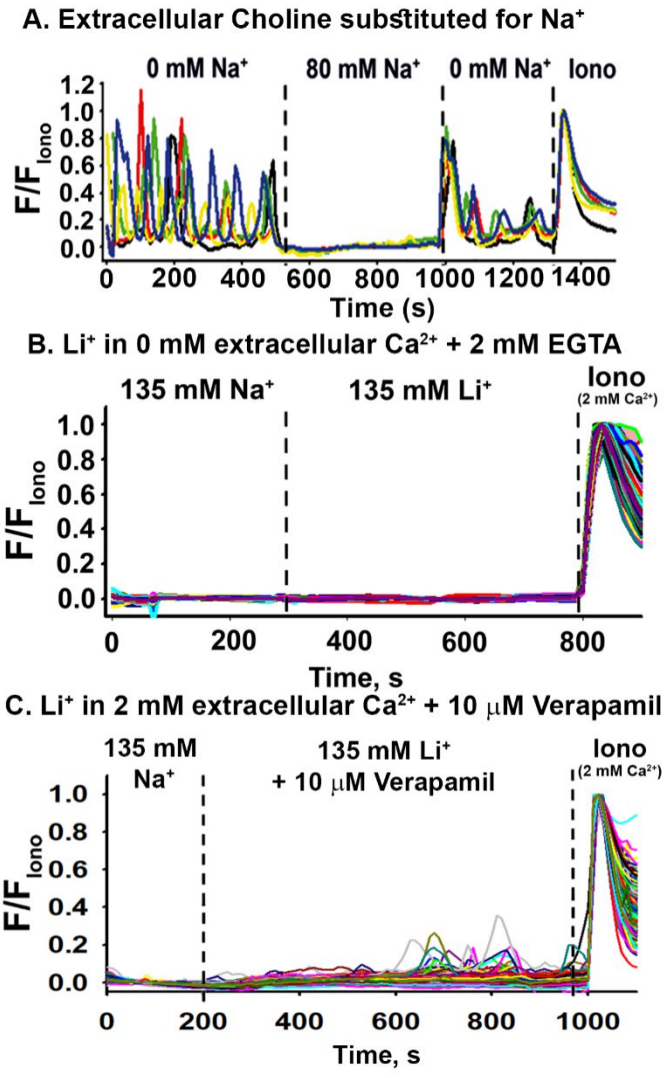


Figure Supplementary 6: Na^+ leak regulates intracellular calcium homeostasis and basal tension in human MSMCs. Related to Figure 3. (A, B, and C) Representative fluorescence traces from human MSMCs loaded with 10 μM Fluo-4 AM. **(A)** Calcium responses in MSMCs by substituting extracellular Choline with Na^+. Reverse experiments of Figure 4B. **(B)** Calcium responses in extracellular 0 mM Ca^{2+} + 2 mM EGTA, when substituting extracellular Na^+ with Li^+ (Values represented in Figure 3E). **(C)** Calcium responses on cells bathed with 10 μM Verapamil, when substituting extracellular Na^+ with Li^+ (Values on Figure 3D). All data were normalized to the fluorescence in 5 μM ionomycin and 2 mM extracellular Ca^{2+} (Iono).