Expanded View Figures

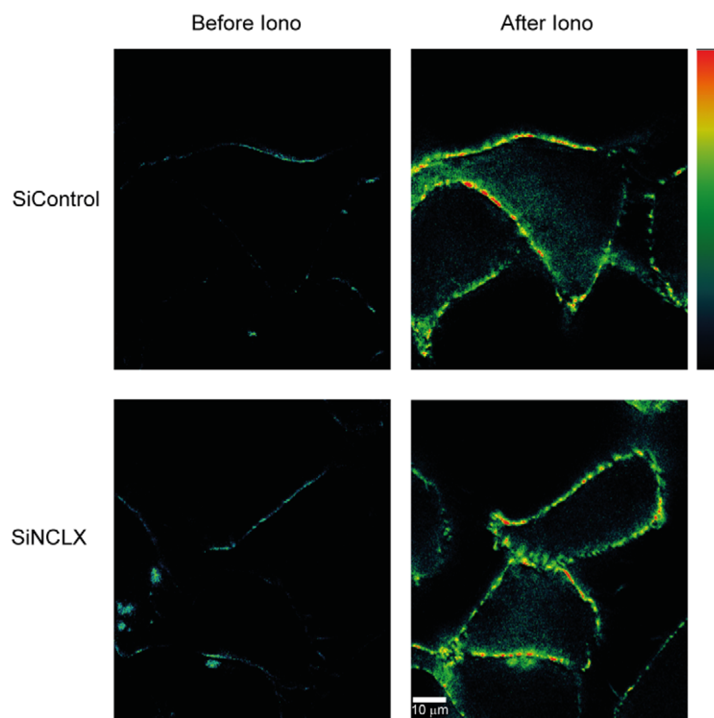

Figure EV1. NCLX knockdown has no effect on Orai1/STIM1 interactions measured with FRET.
HEK293T cells expressing Orai1-CFP and STIM1-YFP were transfected with either control siRNA or NCLX siRNA and effect on Orai1/STIM1 corrected FRET after store depletion with 2.5 μM ionomycin (Iono) were compared. Bar scale: warmer colors indicate high FRET. The scale bar represents 10 μm.

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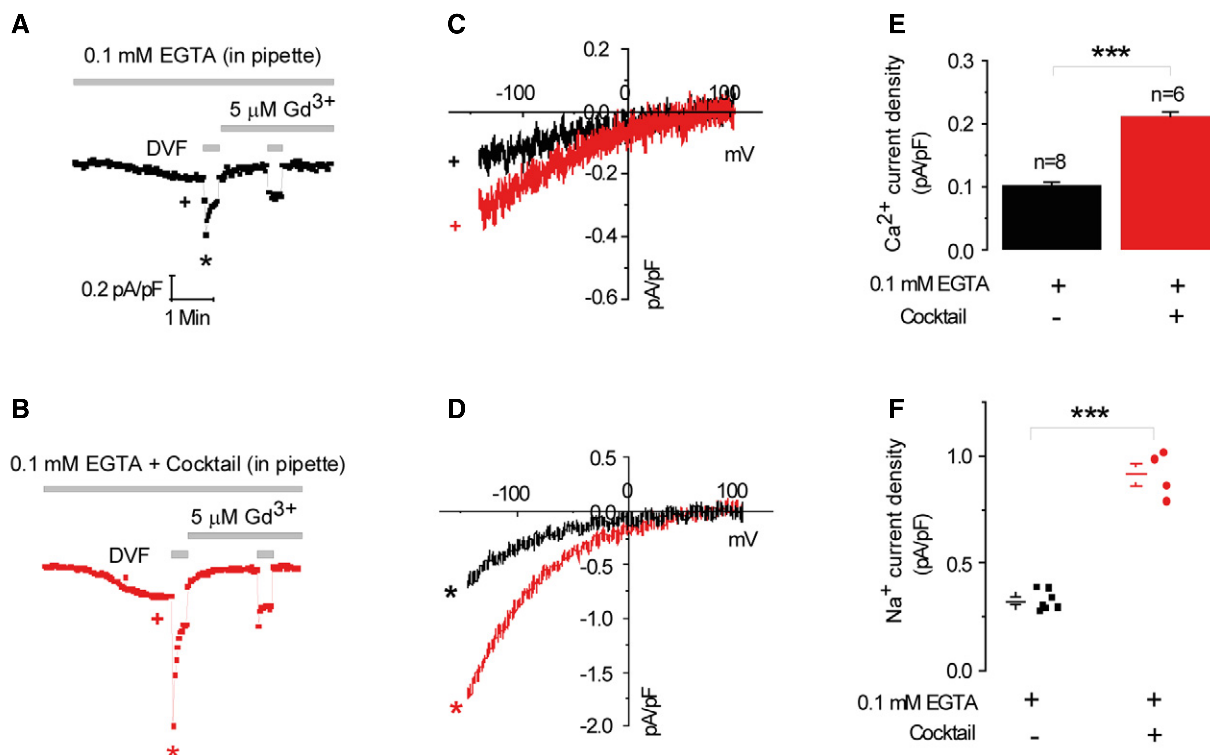

Figure EV2. CRAC channels are enhanced under physiological conditions of energized mitochondria.

A, B Electrophysiological CRAC recordings were performed on HEK293T cells where store depletion was induced by the use of a pipette solution containing 0.1 mM EGTA with IP_3 with (B) and without (A) inclusion of a cocktail to energize the mitochondria. At the end of recordings, 5 μM Gd^{3+} was used to inhibit CRAC currents.

C, D Representative I–V relationships of Ca^{2+} (C) and Na^{+} (D) CRAC in are taken from traces in (A) and (B) where indicated by color-coded asterisks.

E, F Statistical analysis on Ca^{2+} and Na^{+} CRAC currents measured at −100 mV.

Data information: The results are presented as the means ± SEM. ***$P < 1E\text{-}03$. *P*-values indicate the results of an unpaired Student's *t*-test.

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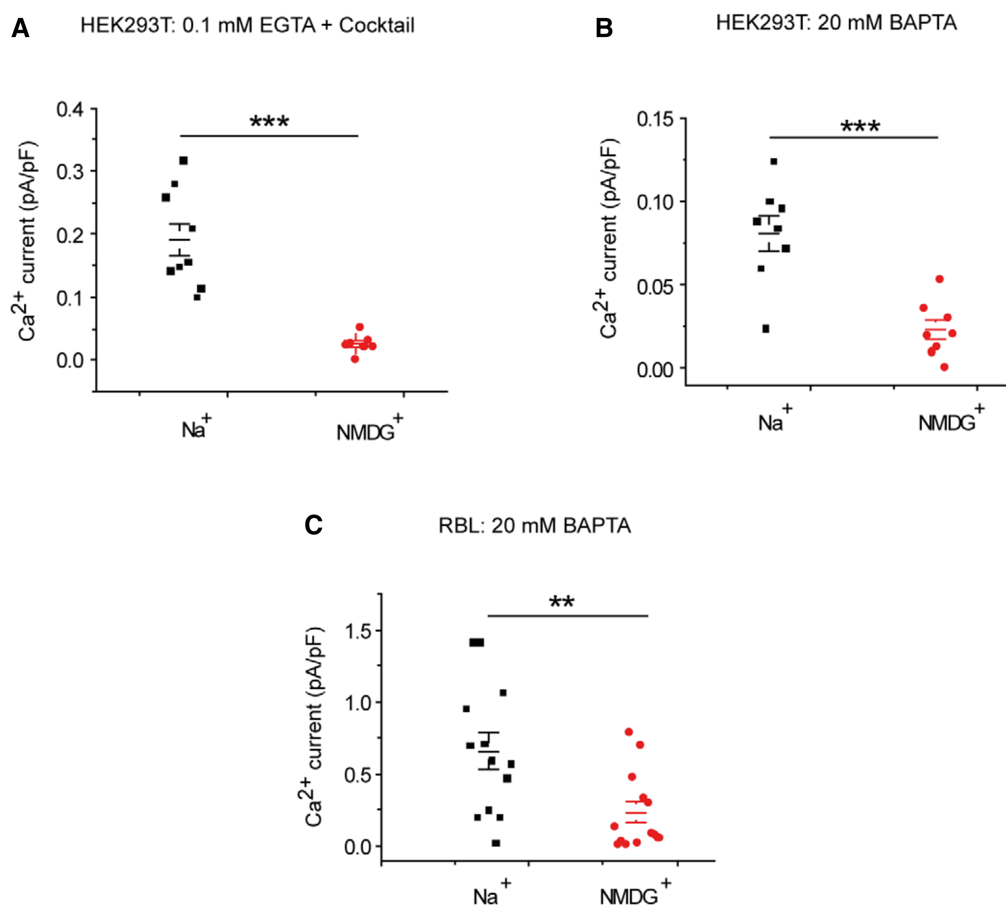

Figure EV3. Substitution of Na^+ by $NMDG^+$ inhibits CRAC currents in HEK293T and RBL cells.

A–C Scatter plots representing Ca^{2+} CRAC currents activated either by dialysis through the patch pipette of a solution containing either 0.1 mM EGTA+IP_3 (A) or 20 mM BAPTA (B) in HEK293T cells, or in RBL cells activated by dialysis of a solution containing 20 mM BAPTA (C). *P*-values (A–C) indicate the results of an unpaired student's *t*-test.

Data information: The results are presented as the means ± SEM. $^{**}P < 0.01$ $^{***}P < 1E\text{-}03$. *P*-values indicate the results of an unpaired Student's *t*-test.

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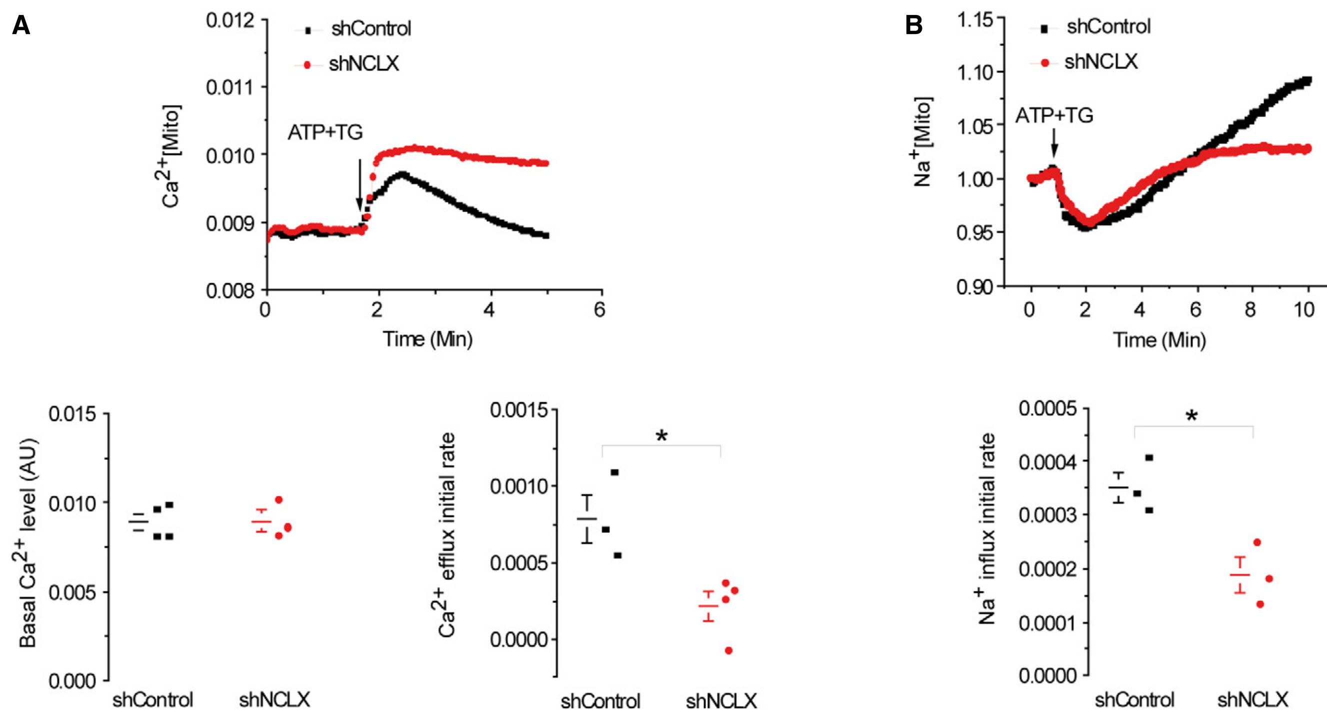

Figure EV4. Knockdown of NCLX expression inhibits mitochondrial Ca^{2+} efflux and Na^+ uptake.

A Upper panel: Mitochondrial Ca^{2+} was monitored in cells expressing the mitochondrial Ca^{2+} sensor RP-mt. Mitochondrial Ca^{2+} transient was evoked by application of ATP and TG, added when indicated. Lower right panel: Rates of mitochondrial Ca^{2+} efflux derived from the upper panel. Lower left panel: The basal Ca^{2+} level (arbitrary units, AU) of shControl (n = 3) versus shNCLX (n = 4).

B Upper panel: Mitochondrial Na^+ uptake in HEK293T cells preloaded with the mitochondrial Na^+ sensor, CoroNa red. Na^+ signals were evoked by ATP and TG added when indicated. Lower panel: Rates of mitochondrial Na^+ influx derived from the upper panel.

Data information: The results are presented as the means ± SEM. *$P < 0.05$. P-values indicate the results of an unpaired Student's t-test.

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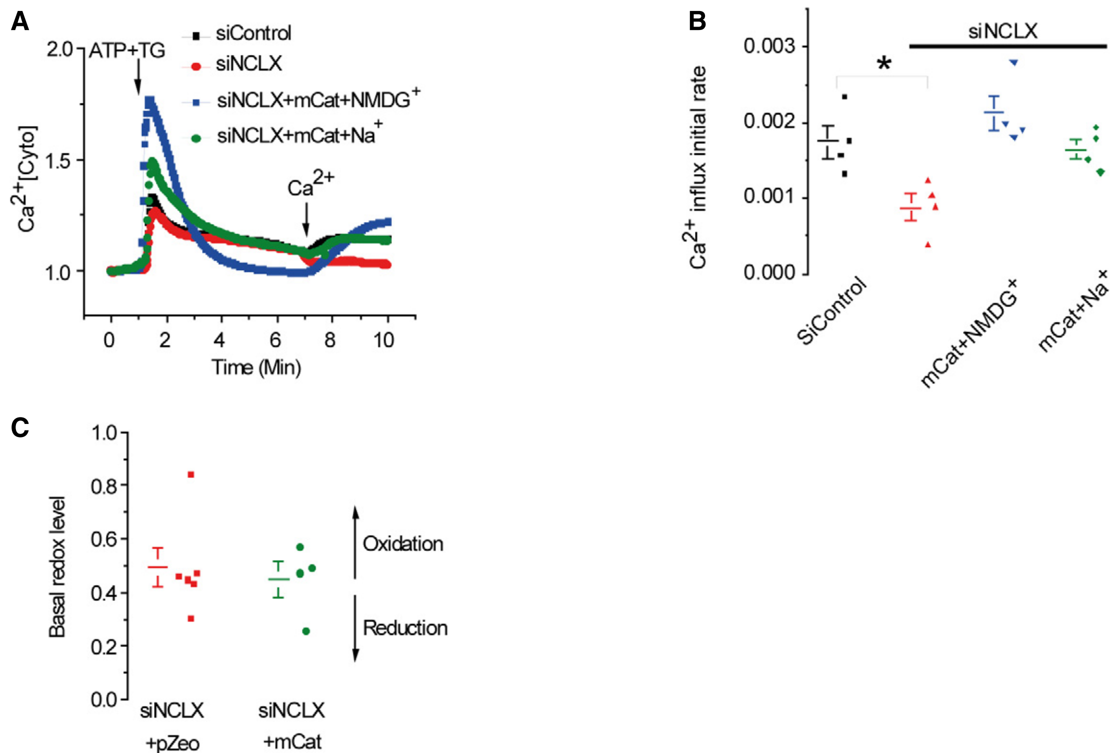

Figure EV5. Na^+ has no effect on SOCE in the presence of m-catalase.

A HEK293T cells were transfected with either siControl or siNCLX with or without m-catalase. Cytosolic Ca^{2+} responses were monitored in HEK293T cells after store depletion by ATP and TG as in Fig 1B in the presence or absence of Na^+ ions.

B, C Averaged rates (means ± SEM) of Ca^{2+} influx in either siControl cells (n = 4), siNCLX (n = 4), siNCLX+m-catalase with Ringer containing Na^+ (n = 4), or $NMDG^+$ (n = 4) are shown in (B). P-values indicate the results of a one-way ANOVA test followed by Tukey *post hoc* analysis. *$P < 0.05$. The basal redox levels of siNCLX+pZeo cells (n = 6) versus siNCLX+m-catalase (n = 4) are shown in (C).

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