Supplementary Information

Amplification of Ca^{2+} Release-Activated Ca^{2+} (CRAC) current by STIM1 and CRACM1 (ORAI1)

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Supplementary Figures

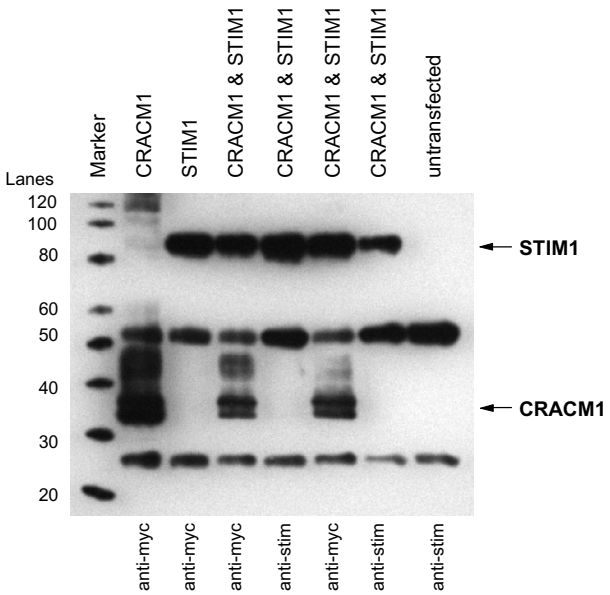

Figure S1: Western blot of combined overexpression of STIM1 and CRACM1. Over-expression of both STIM1 and CRACM1 proteins was confirmed by making whole cell lysates of the co-transfected HEK cells. Since both the proteins are tagged on the C-terminal end with myc and His tags, overexpressed proteins were immunoprecipitated using anti-myc monoclonal antibody, resolved on SDS-PAGE and immunoblotted using anti-His antibody. The co-transfected cells showed the expected 84 KDa band corresponding to STIM1 and a nearly 37 KDa double band corresponding to CRACM1. The STIM1 alone and CRACM1 alone tranfected cells only showed the bands corresponding to STIM1 and CRACM1 respectively. Untransfected control cells did not show any bands corresponding to these two proteins, demonstrating that the anti-His antibody is specifically binding to the overexpressed, tagged proteins. All the lanes had bands corresponding to the heavy and the light chain of the antibodies used for immunoprecipitations at around 25 and 50 KDa. The co-expression strategy is described in the methods section.

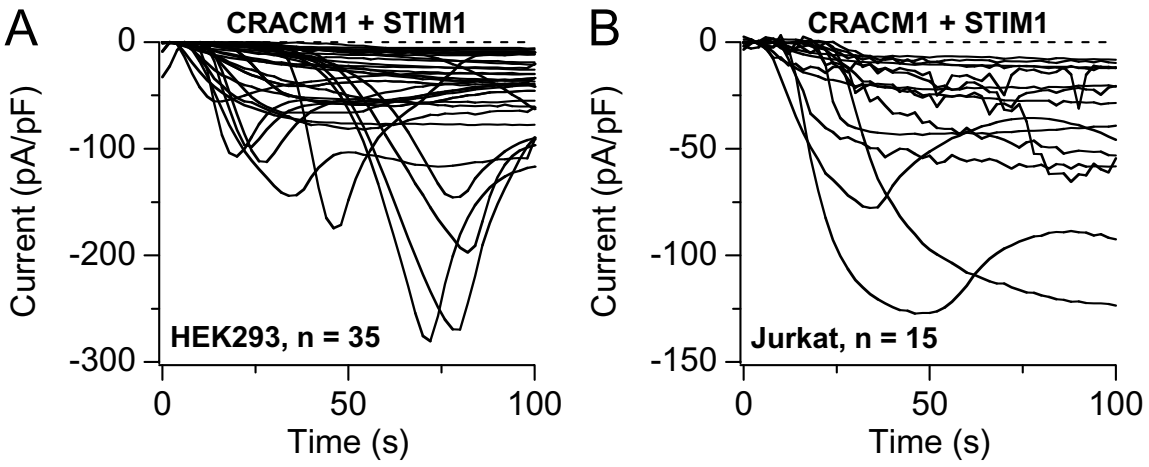

Figure S2: Combined overexpression of STIM1 and CRACM1. (A) Normalized time course of I_{CRAC} in individual HEK293 cells expressing STIM1+CRACM1. Currents of individual cells were measured at –80 mV, normalized by their respective cell size (pF) and plotted versus time. Cytosolic calcium was clamped to near zero with 10 mM BAPTA. Note the delay of current onset in some cells and the inactivation in cells with I_{CRAC} larger than ~50 pA/pF, which is likely due to high $[Ca^{2+}]i$ as the Ca^{2+} buffers are saturated. (B) Normalized time course of I_{CRAC} in individual Jurkat T cells expressing STIM1+CRACM1. Currents were analyzed and plotted as in panel A. Cytosolic calcium was clamped to near zero with 10 mM BAPTA. Note the delay of current onset in some cells and the inactivation in cells with I_{CRAC} larger than ~50 pA/pF.

nature cell biology

METHODS

Electrophysiology. Patch-clamp experiments were performed in the tight-seal whole-cell configuration at 21–25 °C. High-resolution current recordings were acquired using the EPC-9 (HEKA, Lambrecht, Germany). Voltage ramps of 50 ms duration spanning a range of –100 to +100 mV were delivered from a holding potential of 0 mV at a rate of 0.5 Hz over a period of 100–300 s. All voltages were corrected for a liquid-junction potential of 10 mV. Currents were filtered at 2.9 kHz and digitized at 100 μs intervals. Capacitive currents were determined and corrected before each voltage ramp. Extracting the current amplitude at –80 mV from individual ramp current records assessed the low-resolution temporal development of both currents. Where applicable, statistical errors of averaged data are given as mean ± s.e.m. with *n* determinations. Standard external solutions were as follows: 120 mM NaCl, 2.8 mM KCl, 10 mM CsCl, 2 mM $MgCl_2$, 10 mM $CaCl_2$, 10 mM HEPES, 10 mM glucose, at pH 7.2 with 300 mOsm NaOH. In HEK293 cells, 10 mM tetraethylammonium (TEA) was added to suppress delayed rectifier K^+ currents. Standard internal solutions were as follows: 120 mM Cs-glutamate, 8 mM NaCl, 10 mM Cs-BAPTA, 3 mM $MgCl_2$, 10 mM HEPES, 0.02 mM Ins(1,4,5)P_3, at pH 7.2 with 300 mOsm CsOH. As indicated in the figure legends, for some experiments the Ca^{2+} concentration was buffered to 150 nM by 10 mM Cs-BAPTA and 4 mM $CaCl_2$. For passive-depletion experiments, the internal solution was supplemented with Cs-BAPTA in the absence of Ins(1,4,5)P_3 and calcium. In some cells, 2 μM ionomycin was applied for 3 s using a wide-mouth glass pipette. The divalent-free external solution (DVF) was based on the standard external solution but in the absence of $CaCl_2$ and $MgCl_2$ and also contained 10 mM EDTA. Divalent replacement solutions were based on the standard external solution but with 10 mM $CaCl_2$ replaced by either 10 mM $BaCl_2$ or 10 mM $SrCl_2$. 2-aminoethyoxydiphenyl borate (2-APB) was added to the standard external solution at a final concentration of 50 μM. All chemicals were purchased from Sigma-Aldrich Co. (St Louis, MO).

Subcloning and overexpression of CRACM1 and STIM1. Full-length human *CRACM1* and *STIM1* were amplified from human cDNA (PfuTurbo; Stratagene, La Jolla, CA) and subcloned in frame with the carboxy-terminal Myc–His tag in a pcDNA–4TO–Myc–His plasmid (Invitrogen, Carlsbad, CA). The full-length *CRACM1* was re-amplified along with the C-terminal Myc–His tag and subcloned into a pIRES2–EGFP plasmid (Clontech, Mountain View, CA). For overexpression of CRACM1, HEK293 or Jurkat cell lines were transfected with CRACM1–IRES–EGFP using lipofectamine 2000 (Invitrogen). Green cells were analysed for effects of CRACM1 overexpression. For STIM1 overexpression, HEK293 and Jurkat cells were cotransfected with C-terminal Myc–His tagged STIM1 and GFP at a 10:1 ratio, respectively. GFP-expressing cells were analysed by single cell patch-clamp experiments to analyse the effects of STIM1 overexpression. For co-overexpression experiments, *STIM1* was cotransfected with CRACM1–IRES–EGFP plasmid at a ratio of 10:1, respectively and green cells were analysed for the effects of co-expression of the two molecules. In approximately 80% of the green fluorescent HEK293 and Jurkat cells, large currents well in excess of 10 pA pF^{-1} were observed. These cells were presumed to co-overexpress STIM1 and CRACM1–IRES–EGFP.