Supporting Text

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Intracellular Ca^{2+} Measurements. Intracellular Ca^{2+} concentration was measured by ratiometric imaging of fura-2 as described (1). Coverslips with cells grown on them were placed in ion-safe solution A (107 mM NaCl, 7.2 mM KCl, 1.2 mM $MgCl_2$, 11.5 mM glucose, and 20 mM Hepes-NaOH, pH 7.2) and immersed in 2 μM fura-2/acetoxymethylester for 30 min at 20 °C. Subsequently, cells were washed and fura-2 was allowed to de-esterify for 30 min at 20 °C. Approximately 95% of the dye was confined in the cytosol, as determined by the remaining signal after saponin permeabilization (2). Ca^{2+} measurements were performed on an InCyt dual-wavelength fluorescence imaging system (Intracellular Imaging Inc.). Fura-2 was excited at 340 and 380 nm, and the fluorescence emission was monitored at 505 nm. Intracellular Ca^{2+} concentrations are represented by the ratio of the fluorescence intensities measured at 340 and 380 nm from groups of single cells. Measurements are shown as means ± SEM of traces from groups of individual cells (ranging from 9 to 25 cells) and are representative of 3 or more independent experiments.

Electrophysiological Measurements. Studies were performed in HEK Orai1–CFP cells that were transfected with S2–YFP, S1-CT–YFP, or S2-CT–YFP. Conventional whole-cell recordings were undertaken as described (3). Immediately after establishment of the whole-cell configuration, voltage ramps spanning from −100 to +100 mV in 50 ms were delivered from a holding potential of 0 mV at a rate of 0.5 Hz. The intracellular solution contained 145 mM CsGlu, 10 mM Hepes, 10 mM EGTA, 8 mM NaCl, 6 mM $MgCl_2$, 2 mM Mg-ATP (total 8 mM Mg^{2+}), and 3 mM $CaCl_2$, pH 7.2. TRPM7 activity was suppressed by the presence of 8 mM Mg^{2+} and ATP (1). The free Ca^{2+} was 100 nM as calculated by using WEBMAXCLITE (www.stanford.edu/~cpatton/downloads.htm). The extracellular solutions contained 145 mM NaCl, 10 mM $CaCl_2$, 10 mM CsCl, 2 mM $MgCl_2$, 2.8 mM KCl, 10 mM Hepes, and 10 mM glucose, pH 7.4. A 10-mV junction potential compensation was applied.

Western Analyses. Cells were lysed in chilled Nonidet P-40 buffer [1% (vol/vol) Nonidet P-40, 150 mM NaCl, 50 mM Tris·HCl (pH 8.0), containing 100 μM phenylmethylsulfonyl fluoride and Sigma protease inhibitor mixture I] followed by incubation for 30 min at 4 °C and subsequent centrifugation (18,000 × g, 20 min at 4 °C). The protein content of the supernatants was quantified by using Bio-Rad DC protein assay kits. Protein extracts (15 μg/lane of each) were resolved on 8% SDS-polyacrylamide gels and electroblotted onto Bio-Rad Immun-Blot PVDF membranes. After transfer, the PVDF membranes were blocked (1 h, room temperature) in Tris-buffered saline-Tween 20 (TBST; 10 mM Tris·HCl, pH 8.0, 150 mM NaCl, 0.1% Tween 20) containing membrane blocking agent (5%) (Amersham Biosciences) and subsequently incubated with corresponding primary antibodies (1 h, 22 °C). Membranes were washed 2 times (7 min) in TBST and incubated with secondary antibody (30 min, IgG conjugated to horseradish peroxidase). Subsequently, membranes were washed 3 times (5 min) in TBST followed by a single wash (5 min) in Tris-buffered saline (TBS; 150 mM NaCl, 10 mM Tris·HCl, pH 8.0). Peroxidase activity was visualized with a ECL kit as according to the manufacturer's instructions (Amersham Biosciences).

Fluorescence Imaging and FRET Measurements. Experiments were performed in HEK293 cells stably expressing either Orai1–CFP or empty vector (pIRES) that were transiently transfected with S1-CT–YFP or S2-CT–YFP. Fluorescence was examined with a Leica DMI 6000B fluorescence microscope equipped with CFP ($436_{Ex}/480_{Em}$) and YFP ($500_{Ex}/535_{Em}$) filters controlled by Slidebook Software (Intelligent Imaging Innovations). All images were obtained at room temperature with 63× oil objective (N.A. 1.4; Leica), and image analyses were performed with Slidebook software. CFP ($436_{Ex}/480_{Em}$) and $FRET_{raw}$ ($436_{Ex}/535_{Em}$) were monitored at a rate of 0.1 Hz. Two-channel corrected FRET was calculated based on the following formula: $FRET_c = (F_{raw} - F_d/D_d \times F_{CFP})/F_{CFP}$, where $FRET_c$ represents the corrected total amount of energy transfer, F_{raw} represents fluorescence measured through the CFP/YFP ET filter cube, F_{CFP} represents measured CFP fluorescence, and F_d/D_d represents measured bleed-through of CFP through the YFP filter (0.592248). Fluorescence images and FRET analyses shown are typical of at least 3 separate experiments.

1. Soboloff J, et al. (2005) Role of endogenous TRPC6 channels in Ca^{2+} signal generation in A7r5 smooth muscle cells. *J Biol Chem* 280:39786–39794.
2. Ma HT, et al. (2000) Requirement of the inositol trisphosphate receptor for activation of store-operated Ca^{2+} channels. *Science* 287:1647–1651.
3. Soboloff J, et al. (2006) Orai1 and STIM reconstitute store-operated calcium channel function. *J Biol Chem* 281:20661–20665.