Supporting Information

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SI Methods

Cell Culture, RNAi Transfection, and Reagents. Conditions for culturing and transfecting of HSG and HEK293 cells were as previously described (1, 2). siRNA duplexes were designed targeting the coding sequence of human STIM1: (a) ^{577}AAGGCTCTGGATACAGTGCTC^{597} (STIM1-SiRNA1) and (b) ^{850}AAGAAGCTGCGCGATGAGATC^{870} (STIM1-SiRNA2); and Cav1: (a) ^{221}AACCAGAAGGGACACACAGTT^{241} (Cav1-SiRNA1) and (b) ^{505}AATGTCCGCA-TCAACTTGCAG^{525} (Cav1-SiRNA2). A FITC-conjugated non targeting (NT) SiRNA duplex was used as control. All siRNA duplexes were synthesized from Qiagen. Lipofectamine RNAiMAX (Invitrogen) was used for siRNA transfection while Lipofectamine 2000 was used for other plasmids. Cells were typically used 48 h post-transfection. Untagged STIM1 expression vector was from OriGENE Technology. pNFkB-Luc and pRL-TK were obtained from Clontech. For vector control, pcDNA3.1 (Invitrogen) empty plasmid was used. For adenoviral expressions, cells were infected with a MOI of 2–5 pfu of AdTRPC1 and AdCav1 (Vector Biolabs). Antibodies used were anti-TRPC1 (1, 2), anti-Cav-1 (polyclonal/monoclonal, BD Biosciences) and (monoclonal, Santa Cruz Biotechnology), anti-STIM1 (BD Biosciences, Abnova, and Cell Signaling Technologies), anti-HA (Santa Cruz Biotechnology and clone-3F10, Roche), anti-NF-κB-p65, anti-actin, anti-GAPDH (Santa Cruz Biotechnologies or Calbiochem), anti-transferin receptor (Zymed Laboratories), rhodamine- or FITC-linked secondary antibody (Jackson Laboratories), and all HRP-conjugated secondary antibodies (Pierce). All other reagents used were of molecular biology grade obtained from Sigma chemicals unless mentioned otherwise.

Immunoprecipitation, Isolation of Detergent-Resistant Membranes, and Western Blotting. Immunoprecipitation was carried out as described earlier (1, 2). Following stimulation cells were lysed and used for immunoprecipitation. Detergent-resistant membrane raft domains (R) and soluble fractions (S) were isolated from HSG cells as mentioned earlier (1). Proteins were resolved in 4–12% NuPAGE gels followed by Western blotting with the desired antibodies.

Cell Surface Biotinylation. Cells were treated as required and incubated for 20 min with 1.5 mg/mL Sulfo-NHS-LC-Biotin (Pierce) in 1× PBS (pH 8.0) on ice (3). Following biotin labeling cells were washed and solubilized. Biotinylated proteins were pulled down with NeutrAvidin-linked beads (Pierce) and detected by Western blotting. Band intensities of surface proteins were obtained using Quantity One 4.6.5 1D-analysis software (Bio-Rad).

Fluorescence Resonance Energy Transfer (FRET). Acceptor bleaching method was used to measure FRET. Energy transfer efficiency ($FRET_{eff}$) was calculated using the equation: $FRET_{eff} = (D_{post} - D_{pre})/D_{post}$ where D_{post} and D_{pre} are the fluorescence intensity of the donor after and before acceptor photobleaching respectively. Cells were transfected with CFP-Cav1 and YFP-TRPC1 pair, or CFP and YFP empty vectors as controls. Regions of interest (ROI) were marked along the plasma membrane and average $FRET_{eff}$ was calculated in these regions using the FRET wizard in the Leica Confocal Software (Leica Microsystems).

TIRF Microscopy. An Olympus IX81 motorized inverted microscope (Olympus) was used as described previously (2) using 514 and 447 nm lasers for excitation of YFP and CFP respectively, a TIRF-optimized Olympus Plan APO 60× (1.45 NA) oil immersion objective and Lambda 10–3 filter wheel (Sutter Instruments) containing a 480-band pass filter (BP 40 nm) and a 525-band pass filter (BP 50 nm) for emission. Images were collected using a Hamamatsu EM C9100 camera (Hamamatsu) and MetaMorph imaging software (Molecular Devices).

$[Ca^{2+}]_i$ Measurements. Measurements were performed by imaging Fura-2 loaded cells using the Olympus IX50 microscope and Polychrome 4 (TILL Photonics) system. Images were acquired using a Photometrics CoolSNAP HQ camera (Photometrics) and the MetaFluor software (Molecular Devices).

Electrophysiological Measurements. Whole cell-attached patch clamp measurements was performed using Axopatch 200B amplifier (Molecular Devices) as described previously (2). Patch pipette resistance was 3 to 6 mΩ filled with the following solution: 145 mM cesium methane-sulfonate, 8 mM NaCl, 10 mM $MgCl_2$, 10 mM HEPES, 10 mM EGTA, pH 7.2 (CsOH). SOCE was activated by including IP_3 (10 μM) in the pipette solution (as indicated in the figures). Standard external solution contained: 145 mM NaCl, 5 mM CsCl, 1 mM $MgCl_2$, 10 mM HEPES, 10 mM glucose, pH 7.4 (NaOH), containing 10 mM $CaCl_2$. Development of the current was assessed from the current amplitudes at potentials of −80 mV and +80 mV recorded during voltage ramps ranging from −90 to 90 mV (over a period of 1 s, imposed every 4 s) from a holding potential of 0 mV, and digitized at a rate of 1 kHz. A liquid-junction potential of less than 8 mV was not corrected, capacitative currents and series resistance were determined and minimized. The first ramp was used for leak subtraction in the subsequent current records.

Luciferase Reporter Assays. NF-κB driven luciferase reporter assays were performed as mentioned earlier (4). HSG cells were transfected with a reporter vector mixture containing pNF-κB-Luc and pRL-TK (used as internal control). Cells were split and plated on 24-well dish post transfection. Eighteen hours post-seeding, cells were re-transfected with the desired STIM1 construct with or without AdCav1. Twenty-four hours post-transfection, cells were stimulated with 10% FBS in complete MEM for 6 h, following serum starvation, and respective luciferase activities were measured as per the manufacturer's instructions (Promega).

Cell Proliferation. HSG cells transfected as required were synchronized, stimulated with 10% FBS and then pulsed with BrdU for 2 h before BrdU incorporation was measured as per manufacturer's instructions (Roche). Alternatively, cell proliferation on asynchronously growing HSG cells was performed by MTT assay as described earlier (4).

Statistics. Data analysis was performed using Origin 7.0 (OriginLab). Statistical comparisons were made using one way ANOVA. Experimental values are expressed as means ± SD or SEM. Differences in the mean values were considered to be significant at $P < 0.05$.

1. Pani B, et al. (2008) Lipid rafts determine clustering of STIM1 in endoplasmic reticulum-plasma membrane junctions and regulation of store-operated Ca^{2+} entry (SOCE). *J Biol Chem* 283:17333–17340.
2. Ong HL, et al. (2007) Dynamic assembly of TRPC1-STIM1-Orai1 ternary complex is involved in store-operated calcium influx. Evidence for similarities in store-operated and calcium release-activated calcium channel components. *J Biol Chem* 282:9105–9116.
3. Singh BB, et al. (2004) VAMP2-dependent exocytosis regulates plasma membrane insertion of TRPC3 channels and contributes to agonist-stimulated Ca^{2+} influx. *Mol Cell* 15:635–646.
4. Pani B, et al. (2006) Up-regulation of transient receptor potential canonical 1 (TRPC1) following sarco(endo)plasmic reticulum Ca^{2+}-ATPase 2 gene silencing promotes cell survival: A potential role for TRPC1 in Darier's disease. *Mol Biol Cell* 17:4446–4458.

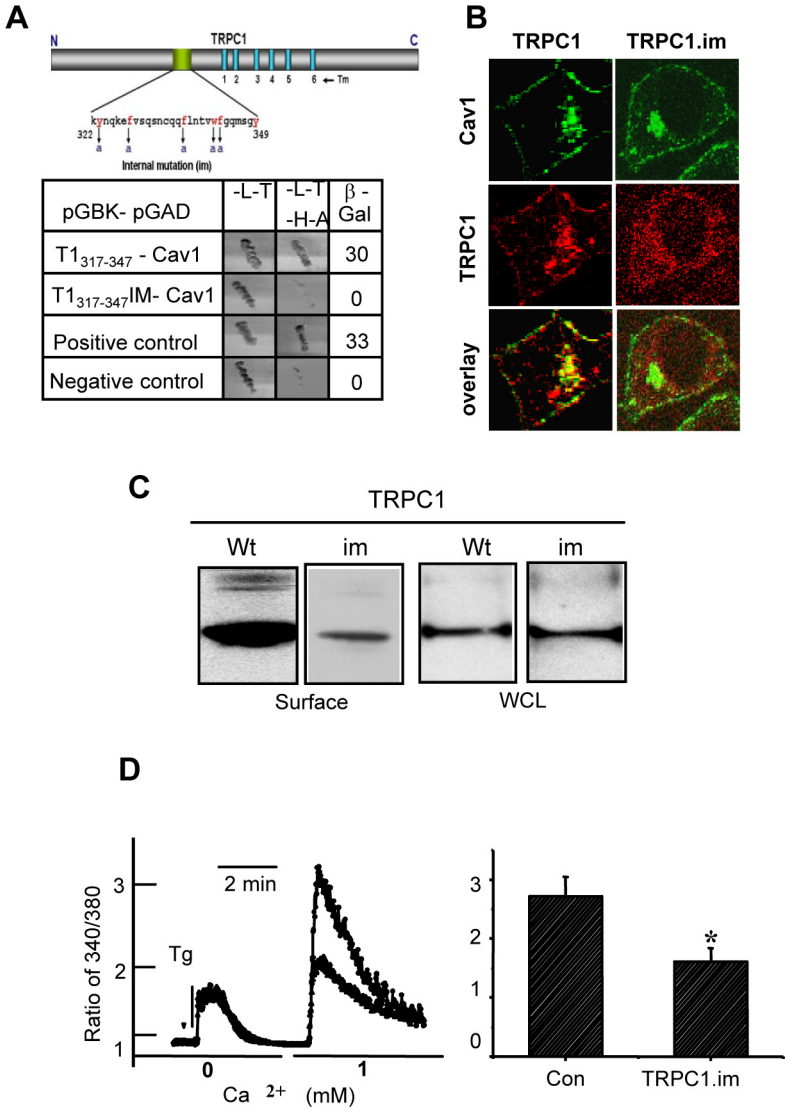


Fig. S1. (*A*) TRPC1 and Cav1 interacting domain was identified using yeast two-hybrid. Amino acids 317–347 in N-terminal region of TRPC1 displayed interaction, whereas mutation of the aromatic aa to alanine (TRPC1-im, upper panel) resulted in loss of interaction with Cav1. (*B*) Confocal microscopy on HSG cells expressing wt-TRPC1 (TRPC1) or mutated TRPC1 (TRPC1-im) using anti-HA antibody (tag on both proteins). Endogenous Cav1 was detected using anti-Cav1 antibody. (*C*) Cell surface labeling of wt-TRPC1 and TRPC1.im in HSG cells by biotinylation with 0.5 mg/mL sulfo-NHS-LC biotin (left blot) and pull down with avidin (surface), input proteins are shown in lanes in WCL (IB: anti-HA). (*D*) Thapsigargin (Tg) stimulated Ca^{2+} mobilization in control and TRPC1.im expressing HSG cells, bar graph, quantitation of the data from more than 200 cells. *, $P < 0.05$ indicates values significantly different from control.

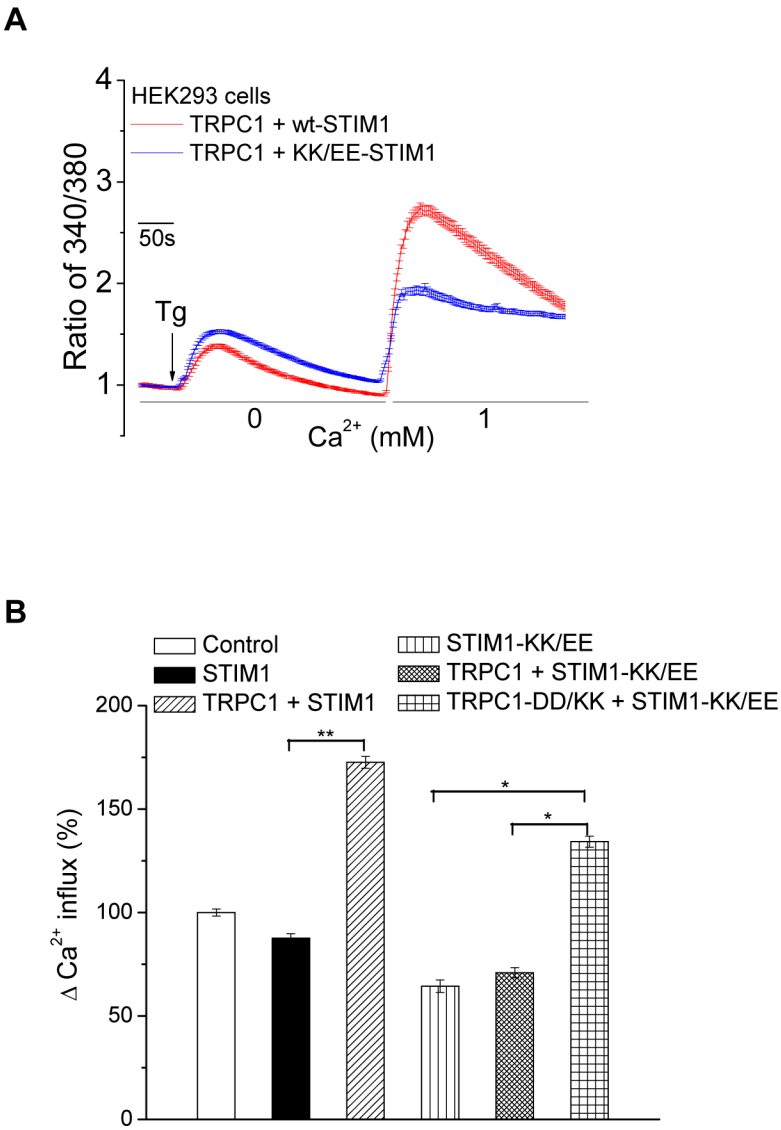


Fig. S2. (*A*) TRPC1 was expressed with wt-STIM1 or STIM1-KK/EE in HEK293 cells. Tg stimulated Ca^{2+} changes were measured as described in the text. (*B*) Bar graph shows the relative changes in SOCE, in control cells and cells expressing either wt-STIM1 or STIM1-KK/EE alone, wt-TRPC1 with STIM1 or STIM1-KK/EE and TRPC1-DD/KK with STIM1-KK/EE. **, $P < 0.05$ indicates values significantly different from STIM1-expressing cells. **, $P < 0.05$ indicates values significantly different from cells expressing STIM1-KK/EE alone or with wt-TRPC1.

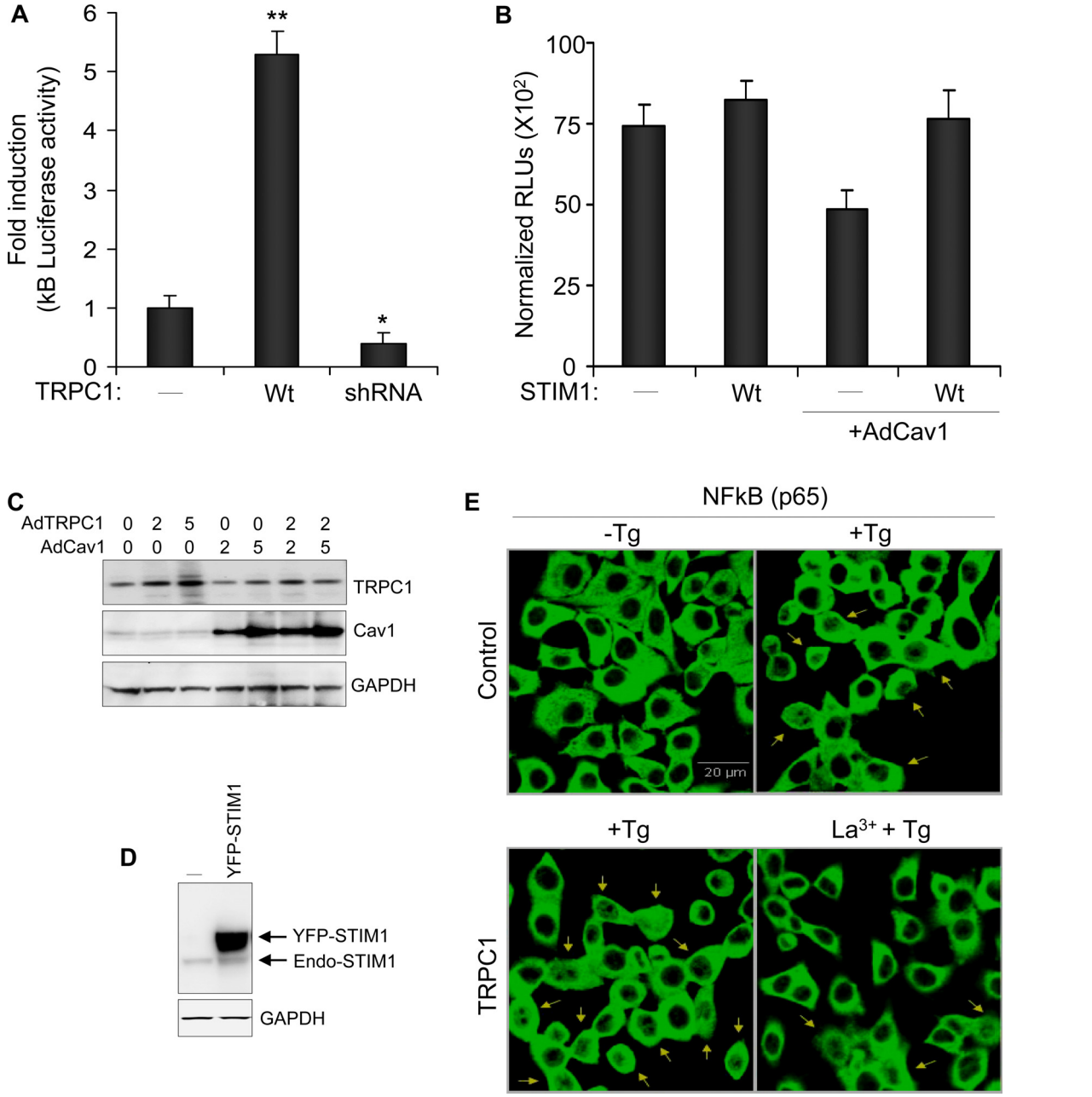
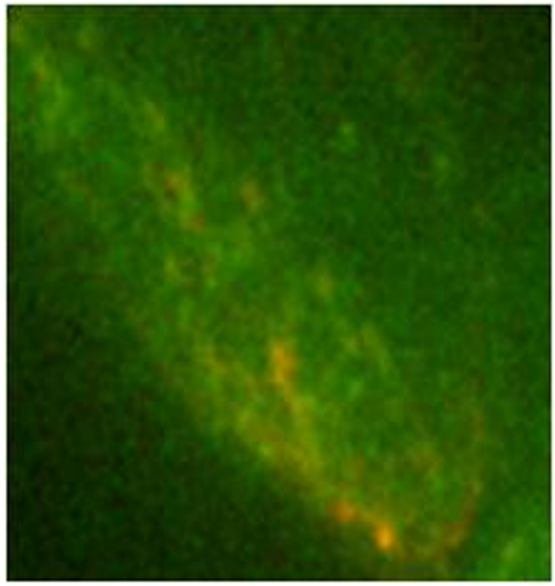
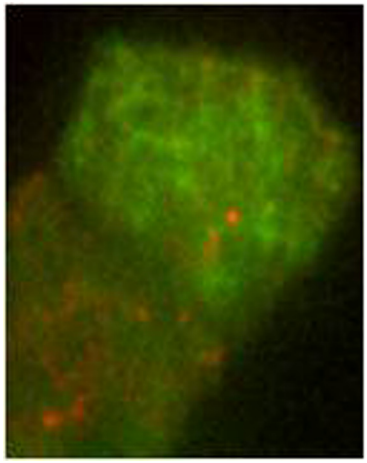


Fig. S3. (*A*) NF-κB driven Luciferase reporter activity in HSG cells expressing TRPC1 or TRPC1-ShRNA. Normalized relative luciferase units (fold induction) are plotted as mean ± SD. **, $P < 0.01$ indicates values significantly different from control cells without overexpression and *, $P < 0.05$ indicates values significantly different from TRPC1-expressing cells. (*B*). Basal (unstimulated) Luciferase reporter activity in HSG cells expressing STIM1, with or without Cav1 (AdCav1, 2MO1). (*C*) Western blots indicating expression levels of TRPC1 and Cav1 under conditions described in (Fig. 5*A*) and exogenous expression of YFP-STIM1 is shown in (*D*). Individual antibodies used for Western blots are indicated in the figure. GAPDH was used as a loading control. (*E*) Confocal images showing nuclear translocation (yellow arrows) of NF-κB (p65) in HSG cells under various conditions as labeled in the figure.



Movie S1. The movie shows an enlarged section of HSG cells expressing CFP-STIM1 (red) and YFP-TRPC1 (green) stimulated with Tg (added in fifth frame after start of movie). Co-localization of YFP-TRPC1 with STIM1-puncta is seen after stimulation. The time lapse covers a period of about 5 min (QuickTime, 3 MB).

Movie S1 (MOV)



Movie S2. The movie shows an enlarged section of cell expressing CFP-STIM1 (red), YFP-TRPC1 (green), and HA-Cav1 stimulated with Tg (added in the fifth frame). The time lapse covers a period of about 5 min. STIM1 puncta (red) are seen, but it was not colocalized with TRPC1 (QuickTime, 2.5 MB).

Movie S2 (MOV)