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Supplemental Material to:

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A plasma membrane-targeted cytosolic domain of STIM1 selectively activates ARC channels, an arachidonate-regulated store-independent Orai channel

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Supporting Information:

SI Methods

Electrophysiology Solutions. For the Flp-In HEK293 cells, the standard extracellular solution contained 140 mM NaCl, 5 mM CsCl, 1.2 mM $MgCl_2$, 10 mM $CaCl_2$, 30 mM glucose, and 10 mM Hepes (pH 7.4). The standard internal (pipette) solution contained 140 mM $CsC_2H_3O_2$, 3.5 mM $CaCl_2$, 3.72 mM $MgCl_2$, 10 mM EGTA, and 10mM Hepes (pH7.2). The calculated free Ca^{2+} concentration was 100 nM. For activation of store-operated CRAC channels, this internal solution was changed to 140 mM $CsC_2H_3O_2$, 4.17 mM $MgCl_2$, 10 mM EGTA, and 10 mM Hepes (pH 7.2) plus 2 μM adenophostin A. For experiments with the RBL-cells, the standard extracellular saline contained 120 mM NaCl, 2.8 mM KCl, 10 mM CsCl, 2 mM $MgCl_2$, 10 mM $CaCl_2$, 10 mM glucose, 10 mM Hepes (pH 7.4), and the standard internal (pipette) solution contained 120 mM Cs-glutamate, 8 mM NaCl, 3 mM $MgCl_2$,10 mM Cs-BAPTA, 10 mM Hepes (pH7.2) plus 2 μM adenophostin A.

Constructs. cDNA encoding human STIM1 was obtained from Dharmacon Inc., (Chicago, IL, USA), and modified to render it siRNA-resistant as described. To generate the Lck-STIM1-C construct, two oligonucleotides (sense and antisense) were generated to prepare an insert coding for the plasma membrane targeting sequence of the Lck kinase (MGCGCSSHPE) followed by a hydrophobic linker (TKLTEER) which included an EcoR1 site at the beginning and an Acl1 site at the end, in-frame with the STIM1 C-terminal region. Following phosphorylation and annealing of these sequences, the insert was ligated to the STIM1 C-terminus position 251-685 (cloned out using PCR including an Acl1 site at the beginning and a Xho1 site following the stop

codon) and inserted into a pcDNA3.1^{+} backbone (Invitrogen). For experiments requiring an C-terminally eGFP-tagged version (Lck-STIM1-C-eGFP), a phosphorylated and annealed linker (AflII to BamH1) was used to remove the stop codon from the above construct, and to allow sub-cloning into a pEGFP-n3 backbone (Invitrogen) in frame with the eGFP.

STIM1-CT constructs were generated by first removing the Lck domain from either the Lck-STIM1-C or Lck-STIM1-C-eGFP construct, as appropriate, using primers designed to introduce a Kpn1 site and a methionine start codon immediately prior to position 251 and the Pml1 site within the C-terminus. The Kpn1/Pml1 cut PCR product was then ligated to a Kpn1/Pml1 cut Lck-STIM1-C backbone.

The S-to-A mutant Lck-STIM1-C constructs were prepared by designing five oligonucleotides (2 sense and 3 antisense) corresponding to the SacII to Acl1 sites in the Lck-STIM1-C construct modified by changing the residue at position 6 in the Lck domain from a serine to an alanine. The oligonucleotides were phosphorylated, annealed and ligated to the Lck-STIM1-C SacII to Acl1 cut backbone.

For the N-terminally eGFP-tagged STIM1-C-CAAX construct, oligonucleotides were designed to add a CAAX box domain to the STIM1 C-terminus including a AflII site at the 5' end and a BamH1 site at the 3' end. These were phosphorylated, annealed and ligated, along with a Kpn1 to AflII STIM1-CT fragment, to a Kpn1/BamH1 cut pAcGFP1-C1 backbone.

All constructs were confirmed by sequencing of the protein coding regions.

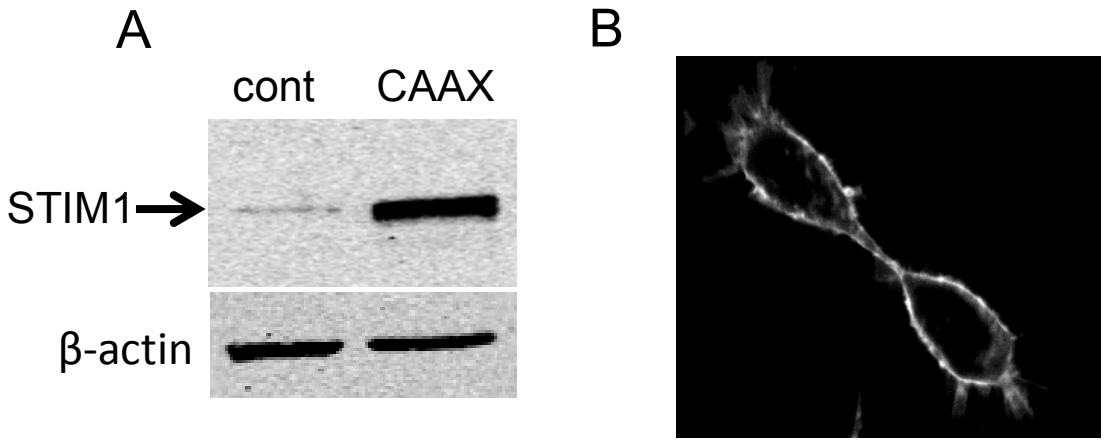

Figure S1. Expression of the STIM1-C-CAAX construct. (*A*) Western blot of cell lysates obtained from control untransfected FlpIn HEK293 cells (cont) and the same cells transfected with the siRNA-resistant STIM1-C-CAAX construct (CAAX). The resulting gel was stained with a STIM1 C-terminal pAb (Cell Signaling). The same gel was then stripped and reprobed with a β-actin Ab as indicated. (*B*) Live confocal image of FlpIn HEK293 cells expressing the eGFP-tagged STIM1-C-CAAX construct.

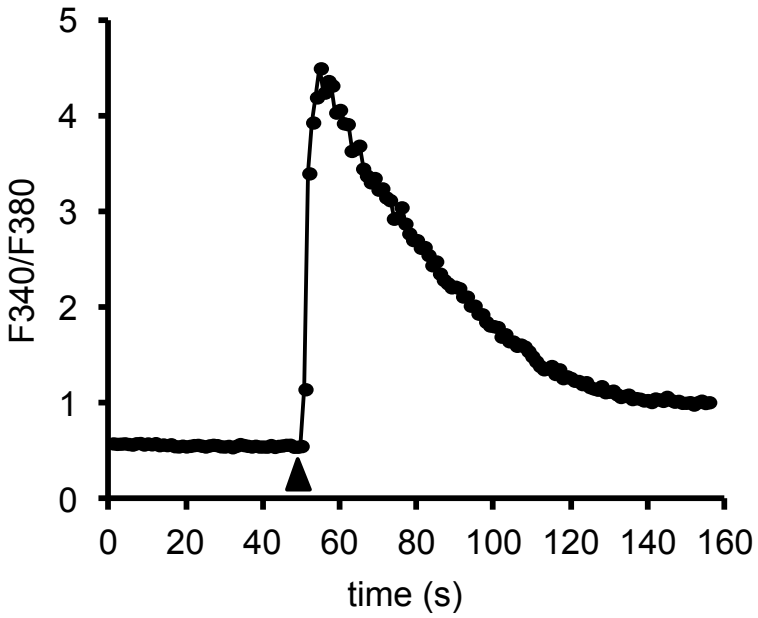

Figure S2. Representative trace of the agonist-induced changes in cytosolic Ca^{2+} concentration in HEK293 cells stably expressing the m3 muscarinic receptor. Cytosolic Ca^{2+} was measured by fura-2 fluorescence ratio imaging, as described. Carbachol (10 μM) was added at the arrow.